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(54) **Sub-region of a plasmodium protein with improved vaccine potential and medical uses thereof**

(57) The present application relates to a sub-region of a *Plasmodium* protein, with improved vaccine potential, and to medical uses thereof, notably for treatment or diagnosis of malaria. The present invention notably provides unstructured or unfolded polypeptides deriving from the PFF0165c protein of *P. falciparum* 3D7. The polypeptides of the invention have a high antigenicity, a high immunogenicity, have a high parasite-killing activity in the ADCI assay, and are strongly associated with clinical protection against malaria, and. The present invention thereby provides a vaccine for the palliative and/or curative treatment of malaria, which is specifically intended for infants, toddlers, children under the age of 5, pregnant women.

EP 2 116 261 A1

Description

FIELD OF THE INVENTION

[0001] The invention pertains to the field of pathogen polypeptidic antigens and nucleic acids coding therefor, and their uses, notably their medical uses, for example, for the curative and/or preventive and/or palliative treatment of a disease related to said pathogen, e.g., by vaccination against said pathogen, and for the diagnosis of a disease related to said pathogen.

[0002] More specifically, the present invention relates to antigenic polypeptidic antigens deriving from *Plasmodium* species sequences, to antibodies directed to said antigens, to nucleic acids coding therefor, to compositions containing said antigens and/or antibodies and/or nucleic acids, as well to method of producing and using same, more specifically in the field of malaria treatment and diagnosis.

BACKGROUND

[0003] Malaria is responsible for over a million deaths a year, with most of the victims being young children. One of the most cost-effective interventions to reduce this toll would be the development of a safe and effective vaccine against *Plasmodium falciparum,* the causative agent of the most severe form of the disease. Individuals living in malaria-endemic regions develop clinical immunity associated with high antibody titers against surface molecules of blood stages (in particular, the merozoite) of the parasite.

[0004] Vaccines against the blood stages of the parasite could reduce the morbidity and mortality, particularly among children. They could also accelerate the acquisition of natural immunity, and help maintain it through constant boosting of the immune response by naturally occurring infections.

[0005] Passive transfer studies have shown that immunoglobulins from semi-immune individuals can confer clinical immunity to individuals exposed to geographically diverse parasite strains.

[0006] Most epitopes recognized by antibodies represent three-dimensional surfaces of an antigen molecule that fit precisely the binding surfaces of the corresponding antibodies (for a review, see Corradin et al., 2007 Endocrine, Metabolic & Immune Disorders - Drug Targets, 7: 259-265).

[0007] These epitopes are classified into distinct groups: linear and structural epitopes. Linear epitopes are made of a continuous unstructured stretch of amino acid residues, while structural protein segment(s) which may be discontinuous (for example, loops) or not. Linear epitopes usually have flexible unfolded conformations and are located in large unstructured loops or terminal protein regions. They can be, in general, mimicked by short protein segments obtained either by protein fragment, or, more simply, by peptide synthesis. Thus, at first sight, linear epitopes do not seem to represent a major technical challenge. However, several estimates that only 10% of the antibodies elicited during an immune response are directed against linear epitopes. Thus most antibodies are raised against discontinuous structural epitopes.

[0008] Therefore, research has up to now mainly focussed on structural or conformation-dependent epitopes, and on the production of short, structurally stable protein segments, which, as isolated peptides, are able to fold into the native structure and thus be recognized by conformation-dependent antibodies. Such structural or conformation-dependent domains notably encompass globular functional domains (such as zinc-fingers, knottins, animal toxins, FGF molecules, chemokines), and structural motifs of protein made of tandem repeats, such as alpha-helical coiled coil domains. For example, WO 2007/020520 in the names of Université de Lausanne and Institut Pasteur describes *Plasmodium* peptidic antigens, such as the P27 antigen (SEQ ID NO: 27 in this PCT international application), which mimick the alpha-coiled coil domain of the native MAL6P1.37 (also referred to as PFF0165c) protein of *Plasmodium falciparum* 3D7.

[0009] By contrast, the natively unfolded or unstructured regions of *Plasmodium* proteins (which are also referred to as IUPs, standing for Intrinsically Unstructured Proteins, see Zhi-Ping Feng et al., 2006 Molecular & Biochemical Parasitology, 150: 256-267) do not fold in any particular unique structure. Natively unfolded or unstructured regions of *Plasmodium* proteins can be identified in the sequence of proteins by bioinformatics analysis (Oldfield et al. 2005 Biochemistry 44: 1989-2000; Linding et al. 2003 Structure (Camb) 11: 1453-1459; Coeytaux and Poupon 2005 Bioinformatics 21: 1891-1900; Dosztanyi et al. 2005 Bioinformatics 21: 3433-3434). At least 15 online services have been established to identify such structures. One of the most widely used services is DisEMBL large-scale sequence analysis (Linding et al. 2003 Structure (Camb) 11: 1453-1459; Zhi-Ping Feng et al., 2006 Molecular & Biochemical Parasitology, 150: 256-267). The DisEMBL comprise three different predictors, Loops/coils, Hot-Loops and REMARK465, which are based on the same algorithm but different training sets, to predict the ordered or disordered state of a residue (cf. http://dis.embl.de/html/help.html).

[0010] In malaria parasites, about 40% of genome-encoded proteins contain natively unstructured regions with segments longer than 50 amino acids. Many of these proteins have a highly hydrophilic amino acid sequence that cannot form a hydrophobic core needed to stabilize a globular structure. Some of these proteins or fragments thereof are

currently being developed as vaccine candidates. These comprise the repeat region of the circumsporozoïte (CS) protein, as well as selected segments of MSP2, MSP3 and GLURP of *P. falciparum*, and the N-terminal and repeat regions of the CS protein of *P. vivax*.

[0011] One of the difficulties in developing peptide antigen from such natively unfolded or unstructured regions of *Plasmodium* proteins is the difficulty of selecting candidates from the large number of predicted unstructured regions found in genomes, and their potential amyloidogenicity.

[0012] The inventors have identified polypeptides deriving from a protein of *Plasmodium falciparum*, which are unfolded or unstructured polypeptides, and which show improved properties with respect to prior art peptides or polypeptides.

[0013] The *P. falciparum* protein, from which the polypeptides of the invention derive, is the MAL6P1.37 (also referred to as PFF0165c) protein of *Plasmodium falciparum* 3D7 (accession number of the protein sequence: XP_966024). This 1103 amino acid-long protein is encoded by chromosome 6 of *P. falciparum* and only is a predicted protein, with no known function yet.

[0014] The present inventors demonstrate that unfolded or unstructured polypeptides, which derive from this predicted protein, have a high antigenicity, a high immunogenicity, and have a parasite-killing activity in the Antibody-Dependent Cellular Inhibition (ADCI) assay that is as high as, or higher than the structured P27 peptide disclosed in WO 2007/020520.

[0015] Furthermore, the total proportion of individuals who, under natural exposure to a malaria parasite, respond by specific IgG1 and IgG3, i.e., the most critical IgG subclasses, is higher for the polypeptides of the invention, such as the P27A than for other antigens, including peptide P27.

[0016] For an illustration of this effect and advantage, please see e.g., the prevalence values indicated in Table 2 in example 2 below, as well as the prevalence values indicated below the diagram of Figure 4. The total proportion of individuals who, under natural exposure to a malaria parasite, respond by specific IgG1 and IgG3, is higher for the polypeptide of the invention P27A (anti-P27A specific IgG1: 86.7% of the individuals; anti-P27A specific IgG3: 82.2% of the individuals) than for other antigens, including peptide P27 (anti-P27 specific-IgG1: 6.7%; anti-P27 specific IgG3: 95.6%).

[0017] Moreover, the polypeptides of the invention are strongly associated with clinical protection against malaria. In human beings under natural exposure to the parasite, the polypeptides of the invention induce specific antibodies (IgG1 and IgG3) that are very strongly associated with a state of resistance to malaria (statistical association, as assessed by a multivariate analysis made in accordance with the methodology described in Roussilhon et al. 2007 PLoS medicine 4(11): 1791-1803, with $p \leq 0.0014$). Parasite-induced antibodies that are specific of the polypeptides of the invention are present in individuals, who resist to malaria and are absent, or are present at low titers, in individuals, who have malaria attack.

[0018] This association with resistance to malaria is much stronger for the polypeptides of the invention than for the prior art antigens, such as the P27 peptide disclosed in WO 2007/020520.

[0019] For an illustration of this effect and advantage, please see e.g., table 3 in example 2 below, showing the F and p values of a multivariate analysis of the association with a state of resistance to malaria, wherein antibodies specific of the P27A polypeptide of the invention have the highest F ratio (28.55, with $p < 0.0001$).

SUMMARY OF THE INVENTION

[0020] The present invention relates to polypeptides, antibodies, hybridomas, nucleic acids, vector, host cells, and uses thereof, as described below.

[0021] More particularly, the present invention relates to polypeptides, which are fragments or variants of the PFF0165c protein of *P. falciparum*. The polypeptides of the invention are sub-fragments of the fragment 1-844 (SEQ ID NO: 6 or 8) of said protein, which have retained the sequence extending from position 223 to position 326 (SEQ ID NO: 10 or 12) of said protein, or are variants of such sub-fragments.

The polypeptide of SEQ ID NO: 10 or NO: 12 has an unfolded or unstructured 3D-arrangement.

[0022] The polypeptides of the invention are efficient in the treatment of a *Plasmodium*-related disease, more particularly of malaria. They are specifically intended for the palliative and/or curative treatment of such a disease, and are specifically suitable to infants, toddlers, children under the age of 5, pregnant women.

[0023] The antibodies of the invention can be used for passive immunotherapy or for the diagnosis of a Plasmodium-related disease, more particularly of malaria.

[0024] The nucleic acids of the invention notably include primers and probes for the detection of *Plasmodium* species, more specifically for the detection of *Plasmodium falciparum* strain(s). They can also be used for the diagnosis of a Plasmodium-related disease, more particularly of malaria.

[0025] Other aspects of the present invention are described in the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026]

FIGURE 1: nucleic acid and protein sequences of the BFFc protein of P. falciparum 3D7.
FIGURE 2: human affinity-purified antibodies and murine antibodies induced by immunization against P27 and P27A specifically stain *Pf*-infected erythrocytes but not sporozoites.
FIGURE 3: Western blots of lysates of infected erythrocytes (human affinity-purified antibodies and murine antibodies induced by immunization against P27 and P27A).
FIGURE 4: prevalence of cytophilic IgG subclass and association with protection were studied against the 14 initial peptides/genes selected. IgG1 = left column (in red); IgG3 = right column (in blue). Geometric means, 95% confidence intervals and prevalence of anti-peptide antibody responses determined in the village of Ndiop.
FIGURE 5: unstructured or unfolded 3D-arrangement of P27A. Structure of the 1-160 fragment of PFF0165c, which contains the polypeptide P27A (SEQ ID NO: 10, shown in black).

The original version of Figures 2, 4 and 5 as filed are in colours; they are available by file inspection.

DETAILED DESCRIPTION OF THE INVENTION

[0027] In the present application, reference is made to the following sequences:

Table 1:

	SEQ ID NO:	
	Nucleic acid	**Protein**
Protein PFF0165c of Plasmodium falciparum 3D7 [mRNA accession number XM_ 960931; protein accession number XP_966024]	1	2
E292G SNP variant thereof, prevalent in certain areas such as Papua New Guinea and Tanzania	3	4
Polypeptide P27AA (fragment 1-844 of the protein PFF0165c of SEQ ID NO: 2)	**5**	**6**
E292G SNP variant thereof, prevalent in certain areas such as Papua New Guinea and Tanzania (fragment 1-844 of the protein PFF0165c of SEQ ID NO: 4)	**7**	**8**
Polypeptide P27A (fragment 223-326 of the protein PFF0165c of SEQ ID NO: 2)	**9**	**10**
E292G SNP variant thereof, prevalent in certain areas such as Papua New Guinea and Tanzania (fragment 223-326 of the protein PFF0165c of SEQ ID NO: 4)	**11**	**12**
Polypeptide P27 (fragment 845-871 of the protein PFF0165c of SEQ ID NO: 2)	13	14
Polypeptide P27A-P27 (fragment 223-844 of the protein PFF0165c of SEQ ID NO: 2)	**15**	**16**
E292G SNP variant thereof, prevalent in certain areas such as Papua New Guinea and Tanzania (fragment 223-844 of the protein PFF0165c of SEQ ID NO: 4)	**17**	**18**
Polypeptide P27A' (fragment 1-326 of the protein PFF0165c of SEQ ID NO: 2)	**19**	**20**
E292G SNP variant thereof, prevalent in certain areas such as Papua New Guinea and Tanzania (fragment 1-326 of the protein PFF0165c of SEQ ID NO: 4)	**21**	**22**
Polypeptide P27A" (fragment 1-640 of the protein PFF0165c of SEQ ID NO: 2)	**23**	**24**
E292G SNP variant thereof, prevalent in certain areas such as Papua New Guinea and Tanzania (fragment 1-640 of the protein PFF0165c of SEQ ID NO: 4)	**25**	**26**

[0028] In the present application, the amino acid positions are computed with respect to the sequence of the full-length PFF0165c protein, which consists of 1103 nucleotides (SEQ ID NO: 2 or 4). Please see Figure 1.
[0029] Therefore, the sequence of SEQ ID NO: 10 is:

HNNNEKNISYDKNLVKQENDNKDEARGNDNMCGNYDIHNERGEMLDKGKSYS
GDEKINTSDNAKSCSGDEKVITSDNGKSYDYVKNESEEQEEKENMLNNKKRS

[0030] The sequence of SEQ ID NO: 12 is:

HNNNEKNISYDKNLVKQENDNKDEARGNDNMCGNYDIHNERGEMLDKGKSYS
GDEKINTSDNAKSCSGDGKVITSDNGKSYDYVKNESEEQEEKENMLNNKKRS

[0031] The invention relates to a **polypeptide**, the amino acid sequence of which is:

i) the sequence of SEQ ID NO: 6 (fragment 1-844 of the sequence of SEQ ID NO: 2);
ii) the sequence of a fragment of said sequence of SEQ ID NO: 6, said fragment having retained the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6; or
iii) a conservative variant, which derives from said sequence of SEQ ID NO: 6 of i) or from said fragment of SEQ ID NO: 6 of ii) by at least one conservative amino acid substitution and/or at least one conservative internal amino acid deletion, provided that said conservative variant has one the following properties:

a. said conservative variant has retained an unstructured or unfolded 3D-arrangement, said unstructured or unfolded 3D-arrangement being as defined below, e.g., as assessed by the Hot-Loops predictor of the DisEMBL-1.4 software, the six parameters of the software being left at their default settings;

b. said conservative variant has not acquired a globular functional domain (such as a zinc-finger, knottin, animal toxin, FGF molecule, chemokine), nor a structural motif of protein made of tandem repeats, such as an alpha-helical coiled coil domain;

c. said conservative variant has retained the property of inducing IgG1 and/or IgG3 antibodies, more particularly, specific IgG1 and/or IgG3, for example when it is injected (e.g., subcutaneously) as an immunogen in a test animal, e.g., a non-human animal, such as a mouse (for example, C3H and/or CB6F1 and/or outbred ICR mice), at a dose of 20 μg with the Montanide® ISA-720 adjuvant;

d. said conservative variant has retained the property of inducing antibodies that are specific of the *Plasmodium*-infected erythrocytes, preferably of *Plasmodium falciparum*-infected erythrocytes, but not of sporozoites, e.g., when it is injected (e.g., subcutaneously) as an immunogen in a test animal, e.g., a non-human animal, such as a mouse (for example, C3H and/or CB6F1 and/or outbred ICR mice) at a dose of 20 μg with the Montanide® ISA-720 adjuvant;

e. in the ADCI assay, said conservative variant has retained an inhibitory effect on *Plasmodium* growth (preferably on the growth of a *Plasmodium falciparum* strain such as the 3D7 strain) that is of at least 90%, preferably of at least 92%, e.g., it has retained the property of:

- o inducing specific IgG1 and/or IgG3, [e.g., by subcutaneous injection of a dose of 20 μg with the Montanide® ISA-720 adjuvant in a test animal, preferably a non-human animal, such as a mouse (for example, C3H and/or CB6F1 and/or outbred ICR mice), collection of the IgG produced 10 days after said injection, and isolation of the IgG1 and/or IgG3, which specifically bind to said conservative variant],
- o wherein said induced IgG1 and/or IgG3 have a SGI value of at least 90%, preferably of at least 92%, in the ADCI assay (i.e., said induced IgG1 and/or IgG3 induce at least 90%, preferably at least 92% of inhibition of a *Plasmodium falciparum* strain such as the *Plasmodium falciparum* 3D7 parasite growth in a monocyte-dependent manner);

f. said conservative variant has retained the property that, in humans under natural exposure to a malaria parasite, the total proportion of individuals having IgG1 and IgG3 antibodies that are specific of this conservative variant is higher than the total proportion of individuals having IgG1 and IgG3 antibodies that are specific of prior art peptides or polypeptides, including peptide P27 (SEQ ID NO: 14); more particularly, the proportion of individuals having IgG1 antibodies that are specific of this conservative variant is higher than the proportion of individuals having IgG1 antibodies that are specific of prior art peptides or polypeptides, including peptide P27 (SEQ ID NO: 14);

g. said conservative variant has retained the property that, in human beings under natural exposure to the parasite, it induces specific antibodies (IgG1 and IgG3) that are very strongly associated with a state of resistance to malaria;

h. said conservative variant has retained the property that parasite-induced antibodies, which are specific of said conservative variant, are present in individuals, who resist to malaria and are absent, or are present at

lower titers, in individuals, who have malaria attack;

i. said conservative variant has retained the sequence of SEQ ID NO: 10, or comprises an ortholog variant sequence of said sequence of SEQ ID NO: 10 thereof, said ortholog variant sequence being the ortholog of SEQ ID NO: 10 in a *Plasmodium falciparum* strain other than the 3D7 strain, said ortholog sequence being comprised in a protein that is the chromosome 6-encoded ortholog of the PFF0165c protein in said other *Plasmodium falciparum* strain, said ortholog variant sequence having preferably at least 98%, preferably at least 99% identity with said sequence of SEQ ID NO: 10 over the entire length of SEQ ID NO: 10 and having a sequence size of 102 to 106 amino acids, preferably of 103 to 105 amino acids, advantageously of 104 amino acids, said ortholog variant sequence being most preferably the E292G variant of the sequence of SEQ ID NO: 10, i.e., the sequence of SEQ ID NO: 12 (see below).

[0032] The sequence of SEQ ID NO: 6 (fragment 1-844 of the sequence of SEQ ID NO: 2), and the fragments of said sequence of SEQ ID NO: 6, which have retained the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6, possess all the properties listed under a) to i) above.

[0033] The present inventors demonstrate that the polypeptides of the invention have a high antigenicity, a high immunogenicity, and have a parasite-killing activity in the ADCI assay. The ADCI assay is well-recognized assay in the field of *Plasmodium.* A detailed description of the protocol of this assay is described in example 1 below, and has also been described in Hasnaa Bouharoun-Tayoun et al. 1995 J. Exp. Med. 182: 409-418.

[0034] Furthermore, the total proportion of individuals who, under natural exposure to a malaria parasite, respond by specific IgG1 and IgG3, i.e., the most critical IgG subclasses, is higher for the polypeptides of the invention, such as the P27A than for other antigens, including peptide P27.

[0035] For an illustration of this effect and advantage, please see e.g., the prevalence values indicated in Table 2 in example 2 below, as well as the prevalence values indicated below the diagram of Figure 4. The total proportion of individuals who, under natural exposure to a malaria parasite, respond by specific IgG1 and IgG3, is higher for the polypeptide of the invention P27A (anti-P27A specific IgG1: 86.7% of the individuals; anti-P27A specific IgG3: 82.2% of the individuals) than for other antigens, including peptide P27 (anti-P27 specific IgG1: 6.7%; anti-P27 specific IgG3: 95.6%).

[0036] Moreover, the polypeptides of the invention are strongly associated with clinical protection against malaria. In human beings under natural exposure to the parasite, the polypeptides of the invention induce specific antibodies (IgG1 and IgG3) that are very strongly associated with a state of resistance to malaria (statistical association, as assessed by a multivariate analysis made in accordance with the methodology described in Roussilhon et al. 2007 PLoS medicine 4(11): 1791-1803, with $p \leq 0.0014$). Parasite-induced antibodies that are specific of the polypeptides of the invention are present in individuals, who resist to malaria and are absent, or are present at low titers, in individuals, who have malaria attack.

[0037] This association with resistance to malaria is much stronger for the polypeptides of the invention than for the prior art antigens, such as the P27 peptide disclosed in WO 2007/020520.

For an illustration of this effect and advantage, please see e.g., table 3 in example 2 below, showing the F and p values of a multivariate analysis of the association with a state of resistance to malaria, wherein antibodies specific of the P27A polypeptide of the invention have the highest F ratio (28.55, with $p < 0.0001$).

[0038] A conservative variant of the invention has at least one, preferably at least two, more preferably at least three, even more preferably at least four, still even more preferably at least five, still even more preferably at least six, still most preferably at least seven, yet still most preferably at least eight of said properties a) to i).

[0039] The preferred combinations of the properties a) to i) listed above comprise the following combinations:

- property a) and/or b); or
- at least one of the properties c) to e), preferably at least two of the properties c) to e), more preferably the three the properties c) and e); or
- at least one of the properties f) to h), preferably at least two of the properties f) to h), more preferably the three the properties f) and h); or
- property a) and/or b), and at least one of the properties c) to e), preferably at least two of the properties c) to e), more preferably the three the properties c) and e); or
- property a) and/or b), and at least one of the properties f) to h), preferably at least two of the properties f) to h), more preferably the three the properties f) and h); or
- at least one of the properties c) to e), preferably at least two of the properties c) to e), more preferably the three the properties c) and e), and at least one of the properties f) to h), preferably at least two of the properties f) to h), more preferably the three the properties f) and h);
- property i); or

- property i) and at least one of the properties a) to h); or
- property i) and at least one of the properties a) to b); or
- property i) and at least one of the properties c) to e); or
- property i) and at least one of the properties f) to h); or
- property i), and at least one of the properties a) to b), and at least one of the properties c) to e); or
- property i), and at least one of the properties a) to b), and at least one of the properties f) to h); or
- property i), and at least one of the properties a) to b), and at least one of the properties c) to e), and at least one of the properties f) to h).

[0040] Most preferably, a conservative variant of the invention has all of the properties a) to i) listed above.

[0041] By internal amino acid deletion, it is herein meant the deletion of an amino acid, which is not the very first amino acid at the N-terminus of the sequence or the very last amino acid at the C-terminus of the sequence.

[0042] Said fragment of SEQ ID NO: 6 can e.g., be:

- the 223-844 fragment, which is of SEQ ID NO: 16 (fragment 223-844 of the sequence of SEQ ID NO: 6); or a sub-fragment thereof, which has retained the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6; or
- the 1-326 fragment, which is of SEQ ID NO: 20; or a sub-fragment thereof which has retained the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6; or
- the 1-640 fragment, which is of SEQ ID NO: 24 (the structure of which is shown in Figure 5), or a or a sub-fragment thereof which has retained the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6.

[0043] Most preferably, said fragment of SEQ ID NO: 6 is the 223-326 fragment, which is of SEQ ID NO: 10 (fragment 223-326 of the sequence of SEQ ID NO: 6).

[0044] Said conservative variant of a fragment of SEQ ID NO: 6 preferably is a variant by at least amino acid substitution.

[0045] More preferably, said at least one amino acid substitution is the E292G substitution, which is observed in certain geographical areas, such as Papua New Guinea and Tanzania.

[0046] The E282G substitution of SEQ ID NO: 6 results in the sequence of SEQ ID NO: 8 (substitution of the amino acid at position 292 in the sequence of SEQ ID NO: 6, i.e., E, by the amino acid G).

[0047] The E282G substitution of SEQ ID NO: 10 results in the sequence of SEQ ID NO: 12.

[0048] The E282G substitution of SEQ ID NO: 16 results in the sequence of SEQ ID NO: 18.

[0049] The E282G substitution of SEQ ID NO: 20 results in the sequence of SEQ ID NO: 22.

[0050] The E282G substitution of SEQ ID NO: 24 results in the sequence of SEQ ID NO: 26.

[0051] Hence, the invention more particularly relates to a polypeptide, the amino acid sequence of which is:

i) the sequence of a fragment of the sequence of SEQ ID NO: 6, said fragment sequence having retained the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6; such as the sequence of SEQ ID NO: 16, 20, 24, 10; or

ii) the sequence of a fragment of the sequence of SEQ ID No: 8, said fragment sequence having retained the sequence (SEQ ID NO: 12) extending from position 223 to position 326 of said sequence of SEQ ID NO: 8; such as the sequence of SEQ ID NO: 18, 22, 26, 12;or

iii) a conservative variant sequence, which derives from said fragment sequences of i) or ii) by at least one conservative amino acid substitution and/or at least one conservative internal amino acid deletion, wherein the resulting conservative variant polypeptide has one, or at least one, preferably at least two, more preferably at least three, even more preferably at least four, still even more preferably at least five, yet still even more preferably at least six, most preferably at least seven, still most preferably at least eight, yet still most preferably all of the a)-i) properties, or one of the preferred combinations of the properties a) to i) as above-described.

[0052] Preferably, a conservative variant of the present invention has retained (and comprises) the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6, or the sequence (SEQ ID NO: 12) extending from position 223 to position 326 of the sequence of SEQ ID NO: 8.

[0053] The sequence of a conservative variant of the invention can e.g., be the fragment of a sequence that is the ortholog of the sequence of SEQ ID NO: 6 or 8 in a *Plasmodium falciparum* strain other than the 3D7 strain, said ortholog sequence being encoded by chromosome 6 of said other *Plasmodium falciparum* strain.

[0054] The sequence of a conservative variant of the invention can e.g., be the fragment of a sequence that is the ortholog of the sequence of SEQ ID NO: 2 or 4 in a *Plasmodium* strain other than a *Plasmodium falciparum* strain, said other *Plasmodium* strain being preferably selected among the strains of *P. vivax, P. ovale, P. malariae, P. berghei, P.*

knowlesi, P. chabaudi, P. yoelii, said ortholog sequence being encoded by chromosome 6 of said other *Plasmodium* strain.

[0055] Preferably, said ortholog protein has a MW of 120-140 kDa, more preferably of 125-135 kDa.

Preferably, the sequence of said ortholog protein is at least 70%, more preferably at least 80%, even more preferably at least 85%, most preferably at least 90% identical to the sequence of the protein PFF0165c of *Plasmodium falciparum* 3D7 (SEQ ID NO: 2 or 4), over the entire length of this PFF0165c protein of *Plasmodium falciparum* 3D7.

[0056] Preferably, a conservative variant of the invention derives from said sequence of SEQ ID NO: 6 or 8, or from said fragment of SEQ ID NO: 6 or 8, by one or several amino acid substitutions, wherein said one or several substitutions do not result in increasing the sequence identity score, said sequence of SEQ ID NO: 6 or 8, or said fragment of SEQ ID NO: 6 or 8, has with respect to human proteins, respectively.

[0057] Preferably, a conservative variant of the invention does not comprise a higher number of Asparagine and Glutamic Acid than said sequence of SEQ ID NO: 6 or 8, or than said fragment of SEQ ID NO: 2 or 4, from which said conservative variant derives.

[0058] Preferably, a conservative variant of the invention does not comprise a higher number of highly hydrophobic residues, preferably not a higher number of Isoleucine (I) and Valine (V), than said sequence of SEQ ID NO: 6 or 8, or than said fragment of SEQ ID NO: 6 or 8, from which said conservative variant derives.

[0059] Preferably, the sequence of a conservative variant of the invention has at least 70% identity with said sequence of SEQ ID NO: 6 or 8, or with said fragment of SEQ ID NO: 6 or 8, from which it derives. Said identity score is computed over the entire length of sequence of SEQ ID NO: 6 or 8, or of said fragment of SEQ ID NO: 6 or 8, respectively.

[0060] Preferably, the sequence of a fragment or conservative variant of the present invention consists of less than 844 amino acids, preferably of 70 to 150 amino acids, more preferably of 80 to 150 amino acids, even more preferably of 85 to 140 amino acids, still even more preferably of 90 to 120 amino acids, most preferably of 100 to 115 amino acids, for example of 104 amino acids.

[0061] The sequence of a fragment of the sequence of SEQ ID NO: 6 may comprise the sequence of SEQ ID NO: 16 or of SEQ ID NO: 20 or of SEQ ID NO: 24, or sub-fragment thereof, which has retained the sequence (SEQ ID NO: 10) extending from position 223 to position 326 of said sequence of SEQ ID NO: 6.

More preferably, the sequence of a fragment of the sequence of SEQ ID NO: 6 consists of the sequence of SEQ ID NO: 10.

[0062] The sequence of a fragment of the sequence of SEQ ID NO: 8 may comprise the sequence of SEQ ID NO: 18 or of SEQ ID NO: 22 or of SEQ ID NO: 26, or sub-fragment thereof, which has retained the sequence (SEQ ID NO: 12) extending from position 223 to position 326 of said sequence of SEQ ID NO: 8.

[0063] More preferably, the sequence of a fragment of the sequence of SEQ ID NO: 8 consists of the sequence of SEQ ID NO: 12.

[0064] As the sequence of SEQ ID NO: 8 is a variant of the sequence of SEQ ID NO: 6, it can also be considered that the sequence of SEQ ID NO: 12 is a more preferred sequence of a conservative variant of the invention.

[0065] A polypeptide of the invention can be isolated from a naturally-occurring (i.e., not engineered by man) sources, e.g., by fragmentation or cleavage from a naturally-occurring protein, or produced by recombinant technology, e.g., from a genetically engineered micro-organism or plant, or by synthesis.

Preferably, said polypeptide is produced by synthesis. Any method of peptide synthesis, which the skilled person finds appropriate to synthesize said peptide, can be used. For example, polypeptides of less than about 120 amino acids, can be produced by stepwise amino acid elongation, e.g., by solid-phase peptide synthesis on a solid support such as beads of polystyrene or polyamide resin (Merrifield 1963. Journal of the American Chemical Society 85: 2149; Atherton, E.; Sheppard, R.C. (1989). Solid Phase peptide synthesis: a practical approach. Oxford, England: IRL Press; Stewart, J.M.; Young, J.D. (1984). Solid phase peptide synthesis, 2nd edition, Rockford: Pierce Chemical Company, 91). Longer polypeptides can be produced by fragment condensation and/or chemical ligation of peptide fragments.

[0066] Advantageously, a polypeptide of the invention is soluble in water.

[0067] A polypeptide of the invention may be chemically modified with respect to the native protein from which it derives, e.g., by glycosylation of the polypeptide.

[0068] The present application also relates to **antibodies,** which are directed to at least one polypeptide of the invention, more particularly which specifically bind to a polypeptide of the invention.

[0069] Preferred antibodies of the invention bind to a polypeptide of the invention, without binding to a human protein, and without binding to another *Plasmodium* protein, polypeptide or peptide, more particularly without binding to the P27 peptide disclosed in WO 2007/020520 (SEQ ID NO: 14 in the present application).

[0070] Antibodies of the invention are useful e.g., for *in vitro* diagnosis and/or for passive immunotherapy, as described below. When it is intended for passive immunotherapy, an antibody of the invention preferably is an Ig1 or an IgG3.

[0071] The antibody may be a polyclonal (e.g., a polyclonal serum) or a monoclonal antibody, including but not limited to fully assembled antibody, single chain antibody, Fab fragment, and chimeric antibody, humanized antibody.

The antibody of present invention may also be used in combination with other therapeutic agents such as proteins, antibodies, and/or with targeting molecules to specifically target a certain cell type, and/or to detection label, such as a

radioisotope to easily detect said antibody.

[0072] Means enabling to produce antibodies are known to the person of skilled in the art.

[0073] Animals can be immunized with a nitrated neurotrophin (or fragment or variant thereof), or an antigenic functional derivative thereof, according to a known method.

[0074] Appropriate animals notably comprise mammals, more particularly non-human mammals, such as rabbit.

[0075] For example, a mammal is injected intraperitoneally or subcutaneously with said nitrated neurotrophin (or fragment or variant thereof), or an antigenic functional derivative thereof.

[0076] Said nitrated neurotrophin (or fragment or variant thereof), or antigenic functional derivative thereof, may be diluted with, or suspended in an appropriate volume of PBS (Phosphate-Buffered Saline), physiological saline or the like.

[0077] An appropriate volume of a standard adjuvant can be mixed with the product, if necessary or desired. Illustrative standard adjuvants notably comprise Freund's (complete or incomplete) adjuvant, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum.*

[0078] It may be useful to conjugate said nitrated neurotrophin (or fragment or variant thereof) to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, by using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydrid or $SOCl_2$.

[0079] The solution is administered to the animals several times, e.g., every 4 to 21 days. In addition, an appropriate carrier can also be used upon immunization with an immunogen.

[0080] Polyclonal antibodies are heterogeneous populations of antibody molecules, which can be derived from the sera of animals immunized with said at least one nitrated neurotrophin (or fragment or variant thereof), or an antigenic functional derivative thereof.

[0081] Monoclonal antibodies (mAb), which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture.

[0082] These include, but are not limited to the hybridoma technique of Kohler and Milstein (1975) Nature 256:495-497; and U.S. Pat. No. 4,376,110, the human B-cell hybridoma technique (Kosbor et al. (1983) Immunology Today 4:72; Cole et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030, and the EBV-hybridoma technique (Cole et al. (1985) Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing a mAb of this invention may be cultivated in vitro or in vivo.

[0083] Production of mAb of the invention notably comprises the collection of immunocytes, such as splenocytes, from an immunized animal, and the fusion of these immunocytes to a fusion partner.

[0084] As a partner cell to be fused with the above immunocyte, a mammalian myeloma cell can be used. Examples of a cell line of a myeloma cell that is preferably used herein include various known cell lines, such as the murine myeloma cell line SP2/0-Ag14, or a fused mouse myeloma / non-malignant B-lymphocyte cell line, such as the ATCC HB8464 cell line.

[0085] Cell fusion of the above immunocytes with myeloma cells can be basically performed according to a known method, for example, the method of Kohler and Milstein *et al.* (Kohler. G. and Milstein, C., Methods Enzymol. (1981) 73, 3-46).

[0086] More specifically, the above cell fusion is performed in a standard nutrition culture solution in the presence of, for example, a cell-fusion accelerator. As a cell-fusion accelerator, for example, polyethylene glycol (PEG), hemagglutinating virus of Japan (HVJ) or the like is used. If desired, an adjuvant such as dimethylsulfoxide can also be used by addition to further enhance fusion efficiency.

[0087] Any ratio of immunocytes to myeloma cells may be set for use herein. For example, it is preferable that the number of immunocytes be 1 to 10 times greater than that of myeloma cells. As a culture solution to be used for the above cell fusion, for example, a RPM11640 culture solution or a MEM culture solution which is appropriate for the growth of the above myeloma cell line, or other standard culture solutions that are used for this type of cell culture can be used. Moreover, a serum fluid such as foetal calf serum (FCS) can be used in combination therewith.

[0088] Cell fusion is performed by mixing sufficiently certain amounts of the above immunocytes and myeloma cells in the above culture solution, adding a PEG (e.g., with an average molecular weight of approximately 1000 to 6000) solution (a general concentration of 30 to 60% (w/v)) pre-heated at approximately 37°C, and then mixing the solution, so as to form target fused cells (hybridomas). Subsequently, an appropriate culture solution is added successively, and then a step of removing the supernatant by centrifugation is repeated, so that reagents for cell fusion or the like that is unfavorable for the growth of the hybridomas is removed.

[0089] The thus obtained hybridomas are selected by culturing the hybridomas in a standard selective culture solution such as a HAT culture solution (a culture solution containing hypoxanthine, aminopterin and thymidine). Culture in the

above HAT culture solution is continued for a time period sufficient for the cells (unfused cells) other than the target hybridomas to die (normally, several days to several weeks). Subsequently, a standard limiting dilution method is conducted, so that screening for and monocloning of hybridomas that produce a target antibody are performed.

[0090] In addition to a method with which the above hybridomas are obtained by immunizing non-human animals with antigens, desired human antibodies having binding activity to said nitrated neurotrophin (or fragment or variant thereof) can also be obtained (see Japanese Patent Publication (Kokoku) No. 1-59878 B (1989)), by sensitizing in vitro human lymphocytes with said nitrated neurotrophin (or fragment or variant thereof), or a functional antigenic derivative thereof, and causing the sensitized lymphocytes to fuse with the human-derived myeloma cells having a permanent division potential.

[0091] The thus prepared hybridomas producing monoclonal antibodies can be passage-cultured in a standard culture solution, or can be stored for a long period in liquid nitrogen.

[0092] One example of a method employed to obtain monoclonal antibodies from the hybridomas involves culturing the hybridomas and obtaining monoclonal antibodies in the culture supernatant according to a standard method. Another method involves administering the hybridomas to mammals that are compatible with the hybridomas to cause them to proliferate, and obtaining monoclonal antibodies in the ascites. The former method is suitable to obtain antibodies of high purity. On the other hand, the latter method is suitable for the mass production of antibodies.

[0093] A monoclonal antibody that can be used in the present invention can be a recombinant monoclonal antibody that is prepared by cloning the antibody gene from the hybridoma, incorporating the gene into an appropriate vector, introducing the vector into a host, and then causing the host to produce the recombinant monoclonal antibodies by genetic engineering techniques (e.g., see Vandamme, A. M. et al., Eur. J. Biochem. (1990) 192, 767-775, 1990).

[0094] In addition to the above host cell, a transgenic animal or plant can also be used to produce a recombinant antibody.

[0095] The present invention also relates to a hybridoma secreting a monoclonal antibody of the invention.

[0096] In addition to the above antibody, artificially altered gene recombinant antibodies such as chimeric antibodies or humanized antibodies can be used for, for example, lowering heteroantigenicity against a human. These altered antibodies can be produced using a known method.

Chimeric antibodies can e.g., be obtained by ligating the DNA encoding the antibody V-region to a DNA encoding a human antibody C-region, incorporating the product into an expression vector, and then introducing the vector into a host to cause the host to produce the antibodies. Using this known method, chimeric antibodies useful in the present invention can be obtained.

[0097] Humanized antibodies are also referred to as reshaped human antibodies, which are prepared by grafting an antibody CDR (complementarity determining region) of a mammal other than a human, such as a mouse, to the CDR of a human antibody. The general gene recombination technique thereof is also known (see European Patent Application Publication EP 125023 and WO 96/02576, or any one of their US counterparts, such as e.g., US 6 068 040).

[0098] An antibody used in the present invention is not limited to the whole molecule, and may be a fragment of the antibody or the modified product thereof, as long as it still binds to at least one nitrated neurotrophin (or fragment or variant thereof) and has retained the capacity of inhibiting and/or blocking the apoptotic effect exerted by said at least one nitrated neurotrophin (or fragment or variant thereof) on motor neurons, and/or the capacity of inhibiting and/or blocking the stimulation and/or induction effect exerted by said at least one nitrated neurotrophin (or fragment or variant thereof) on sensory ganglia.

[0099] Multivalent, preferably bivalent, antibody and a monovalent antibody are included. Examples of the fragment of an antibody include Fab, F(ab')2, Fv, Fab/c having one Fab and a complete Fc, and a single chain Fv (scFv) wherein the Fv of the H-chain or the L-chain is ligated with an appropriate linker. Specifically, an antibody fragment is synthesized by treating the antibody with an enzyme such as papain or pepsin, or genes encoding these antibody fragments are constructed, the genes are introduced into expression vectors, and the genes are then expressed by appropriate host cells (see e.g., Rousseaux, J. et al., Methods in Enzymology (1989) 121, 663-669, and Bird, R. E. et al., TIBTECH (1991) 9, 132-137).

[0100] scFv is obtained by linking the H-chain V-region and the L-chain V-region of antibodies. In the scFv, the H-chain V-region and the L-chain V-region are linked via a linker, or preferably a peptide linker (Huston, J. S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 5879-5883). The H-chain V-region and the L-chain V-region in scFv may be derived from any of those described as antibodies in this specification. As a peptide linker to link the V-regions, for example, any single-stranded peptide comprising 12 to 19 amino acid residues is used.

[0101] A DNA encoding scFv can be obtained as follows. Amplification is performed by the PCR method using as templates the entire or DNA portions encoding desired amino acid sequences (of a DNA encoding the H-chain or the H-chain V-region of the above antibody, and a DNA encoding the L-chain or the L-chain V-region), and using a primer pair that specifies both ends. Amplification is then further performed by a combined use of a DNA encoding a peptide linker portion and a primer pair that specifies to cause both ends to ligate respectively to the H-chain and L-chain.

[0102] Furthermore, once a DNA encoding scFv is prepared, expression vectors containing the DNAs, and hosts

transformed with the expression vectors, can be obtained according to the standard method. In addition, by the use of the host, scFv can be obtained according to the standard method.

[0103] These antibody fragments can be produced using hosts by obtaining the genes thereof in a manner similar to the above method, and then causing the expression of the genes. The "antibody" in the present invention also encompasses these antibody fragments.

While transgenic mammalian cells (e.g., Chinese hamster ovary cells) grown in culture are the industry standard for producing full length mAb, mammalian cells may be less suited for the production of antibody fragments such as Fab or scFv, and prokaryotic expression systems (e.g., *E. coli*) or other eukaryotic expression systems, such as yeast or plant cells, may preferably be used

[0104] Furthermore, the antibody used in the present invention may be a bispecific antibody, which can also be prepared by genetic engineering techniques.

[0105] The antibodies expressed and produced as described above can be isolated from the cells or host animals, and purified to a uniform level. Isolation and purification of the antibodies to be used in the present invention can be performed using affinity columns. An example of a column using a protein A column is a Hyper D, POROS, Sepharose F. F. (Pharmacia). Other standard isolation and purification methods that are employed for proteins may be used, and there is no limitation regarding their use. For example, a chromatography column other than the above affinity column, a filter, ultrafiltration, a method of salting out, dialyses and the like may be appropriately selected and combined for use, so that antibodies can be isolated and purified (Antibodies A Laboratory Manual. Ed Harlow, David Lane, Cold Spring Harbor Laboratory, 1988).

[0106] Chemicals that mimic the function(s) of an antibody can be produced.

[0107] There are several approaches to the structure and manufacture of chemicals that mimic the function(s) of an antibody of the invention.

[0108] One approach utilizes an alternative protein framework, such as cytochrome b562, or structures comprising ribonucleic acids (RNA) (Hsieh-Wilson et al. 1996, Acc. Chem. Res. 29:164-170).

[0109] Unnatural oligomers, such as benzodiazepines, beta-turn mimics, protease inhibitors and purine derivatives have also been tested for their ability to function as antibody mimics.

[0110] Unnatural biopolymers, such as oligocarbamates, oligoureas and oligosulfones, have been proposed as antibody mimics.

[0111] Molecules with some of the recognition properties of antibodies have been created by joining various substituents to scaffolds such as xanthese or cubane, or a calixarene unit. These molecules have multiple peptide loops as the recognition site, but built around the relatively rigid organic framework formed by the scaffold.

[0112] The invention more particularly relates to those antibody mimics that have a capacity of inhibiting and/or blocking the apoptotic effect exerted by a nitrated neurotrophin (or fragment or variant thereof) on motor neurons, and/or of inhibiting and/or blocking the stimulation and/or induction effect exerted by a nitrated neurotrophin (or fragment or variant thereof) on sensory ganglia.

[0113] The invention also relates to methods for the *in vitro* **diagnosis** of a *Plasmodium*-related disease, more specifically malaria in an individual, either by using at least one polypeptide of the invention, or by using at least one antibody of the invention, as defined above, as well as to compositions (more particularly pharmaceutical compositions) and kits comprising at least part of the necessary reagents (polypeptides, antibodies ...) for performing these diagnosis methods.

[0114] The invention relates to a method for the *in vitro* diagnosis of a *Plasmodium*-related disease, more specifically malaria, in an individual suspected to be infected by one or several *Plasmodium* species, more specifically *P. falciparum.* The method of the invention comprises bringing a biological sample from said individual into contact with at least one polypeptide of the invention, under conditions enabling the formation of antigen/antibody complexes between said at least one polypeptide and the antibodies possibly present in said biological sample, and *in vitro* detecting the antigen/antibody complexes possibly formed.

[0115] The diagnosis method may further comprise bringing said biological sample into contact with one or several antigenic peptides originating from other *Plasmodium* antigens, such as LSA-1, LSA-3, LSA-5, SALSA, STARP, TRAP, PfEXP1, CS, MSP-3-1, MSP-3-2, MSP-3-5, MSP-3-6, MSP1, MSP2, MSP4, MSP5, AMA-1, SERP and GLURP, in particular from LSA-3, SERP and GLURP, as well as the antigens disclosed in WO 2007/020520, more specifically the P27 antigen (SEQ ID NO: 14 in the present application) or an ortholog sequence thereof from *P. vivax, P. berghei, P. knowlesi, P. chabaudi, P. yoelii.*

[0116] An alternative method of the invention for the *in vitro* diagnosis of a *Plasmodium*-related disease, more specifically of malaria, in an individual suspected to be infected by a *Plasmodium* species, such as *P. falciparum* comprises bringing a biological sample from said individual into contact with at least one antibody of the invention, under conditions enabling the formation of antigen/antibody complexes between said at least one antibody and the *Plasmodium*, more specifically the *P. falciparum*, antigen(s) possibly present in said biological sample, and in vitro detecting the antigen/antibody complexes possibly formed.

[0117] In the *in vitro* diagnosis methods of the invention, any mean appropriate to the detection of antigen/antibody

complexes can be used, for example an ELISA assay.

[0118] Compositions and kits for the *in vitro* diagnosis of a *Plasmodium*-related disease, more specifically malaria are also contemplated by the present application.

[0119] For example, a diagnosis composition or kit of the invention may comprise at least one polypeptide according to the invention, possibly bound to a support. Such a kit can further comprise reagents for enabling the formation of antigen/antibody complexes between said antigenic polypeptide and the antibodies possibly present in a biological sample, and reagents enabling the *in vitro* detection of the antigen/antibody complexes possibly formed.

[0120] An alternative diagnosis composition or kit of the invention for the *in vitro* diagnosis of a Plasmodium-related disease, more specifically malaria, comprises at least one antibody of the invention, as described above, and, optionally, reagents for enabling the formation of antigen/antibody complexes between said at least one antibody and *Plasmodium* (P27A) antigens possibly present in a biological sample, and, if optionally, reagents enabling the *in vitro* detection of the antigen/antibody complexes possibly formed.

[0121] The application also relates to methods for the **treatment,** more specifically the palliative and/or curative treatment, of a Plasmodium-related disease, more particularly of malaria, as well as to compositions and kits for such methods.

[0122] When a composition of the invention is intended for the treatment of a Plasmodium-related disease, more specifically of malaria, it is a medicament, more specifically an immunogenic composition, more particularly a vaccine composition.

[0123] An immunogenic or vaccine composition of the invention comprises at least one polypeptide of the invention as an immunogen, or at least one antibody or antibody fragment of the invention, as defined above, as passive immunotherapeutic agent.

[0124] The main medical effect of the immunogenic or vaccine composition of the invention is to reduce the morbidity of the malaria disease. The immunogenic or vaccine composition of the invention is mainly a palliative and/or curative composition.

[0125] Indeed, when administered to an animal as immunogens, the polypeptides of the invention induce antibodies that mainly target the blood stages of the *Plasmodium* infection, and not the pre-erythrocytic stage. Therefore, an immunogenic or vaccine composition of the invention does not aim at preventing infection or at destroying the parasites at the pre-erythrocytic stage, but at:

- reducing the morbidity and/or mortality, and/or at
- accelerating the acquisition of natural immunity against the parasite and/or at
- maintaining the natural immunity at a level appropriate that is appropriate to the patient's health.

[0126] An immunogenic or vaccine composition of the invention is intended for any patient in need thereof, more particularly for any patient, whether adult or not, who is suspected to be contaminated by a malaria-related *Plasmodium* species, such as *Plasmodium falciparum*. An immunogenic or vaccine composition of the invention is more specifically intended for infants, toddlers, children under the age of 5, pregnant women.

[0127] The preferred target patients are those from countries, where *P. falciparum* is endemic, especially in sub-Sahara Africa, where it is responsible for at least a million deaths per year.

[0128] Said at least one antigenic polypeptide of the invention can be conjugated with a carrier protein, such as keyhole limpet hemocyanin, horseshoe crab hemocyanin, or bovine serum albumin.

[0129] An immunogenic or vaccine composition of the invention, which comprises at least one antigenic polypeptide of the invention as an immunogen, may further contain at least one vaccination adjuvant, such as:

- Freund's adjuvant, either complete or incomplete; Titermax® gold adjuvant; alum; LPS such as bacterial LPS; gamma-linolenic acid (GLA), such as GLA-57, GLA-27, GLA-58, GLA-59, GLA-60; Montanide® ISA 720;
- Mineral salts, e.g., aluminium hydroxide and aluminium or calcium phosphate gels;
- Oil emulsions and surfactant based formulations, e.g., MF59 (microfluidised detergent stabilised oil-in-water emulsion), QS21 (purified saponin), AS02 [SBAS2] (oil-in-water emulsion + MPL + QS-21), Montanide ISA-51 and ISA-720 (stabilised water-in-oil emulsion);
- Particulate adjuvants, e.g., virosomes (unilamellar liposomal vehicles incorporating influenza haemagglutinin), AS04 ([SBAS4] Al salt with MPL), ISCOMS (structured complex of saponins and lipids), polylactide co-glycolide (PLG);
- Microbial derivatives (natural and synthetic), e.g., monophosphoryl lipid A (MPL), Detox (MPL + M. Phlei cell wall skeleton), AGP [RC-529] (synthetic acylated monosaccharide), DC_Chol (lipoidal immunostimulators able to self organise into liposomes), OM-174 (lipid A derivative), CpG motifs (synthetic oligonucleotides containing immunostimulatory CpG motifs), modified LT and CT (genetically modified bacterial toxins to provide non-toxic adjuvant effects);
- Endogenous human immunomodulators, e.g., hGM-CSF or hIL-12 (cytokines that can be administered either as

protein or plasmid encoded), Immudaptin (C3d tandem array);

- Inert vehicles, such as gold particles.

[0130] Said at least one adjuvant preferably is alum, Montanide® ISA 720, or a GLA.

[0131] An immunogenic or vaccine composition of the invention may contain at least two different adjuvants, e.g., two different adjuvants selected from alum, Montanide® ISA 720 and GLA, more preferably alum and a GLA.

[0132] An immunogenic or vaccine composition of the invention may comprise several polypeptides of the invention, e.g. two or three polypeptides of the invention, or several antibodies or antibody fragments of the invention, e.g. two or three antibodies or antibody fragments of the invention of the invention.

[0133] An immunogenic or vaccine composition of the invention, which comprises at least one antigenic polypeptide of the invention as an immunogen, may comprise at least one anti-*Plasmodium* immunogenic compound other than a polypeptide of the invention, more particularly at least one other protein, polypeptide or peptide having anti-*Plasmodium* immunogenic properties.

[0134] In addition to said at least one polypeptide of the invention, an immunogenic or vaccine composition of the invention may for example comprise at least anti-*Plasmodium* immunogenic protein, polypeptide or peptide, which derives from, or is a fragment of, a *Plasmodium* protein selected from a MSP2 protein of *P. falciparum*, a MSP3 protein of *P. falciparum* (MSP-3-1, MSP-3-2, MSP-3-5, MSP-3-6), a MSP4 protein of *P. falciparum*, a MSP5 protein of *P. falciparum*, a GLURP protein of *P. falciparum*, a LSA protein (LSA-1, LSA-3, LSA-5,) a SALSA protein, a STARP protein, a TRAP protein, a PfEXP1 protein, an AMA-1 protein, a SERP protein, a N-terminal and repeat region of the CS protein of *P. vivax*, the Plasmodium peptides described in WO 2007/020520, more specifically the PFF0165c-derived P27 peptide disclosed under SEQ ID NO: 27 in WO 2007/020520, which is herein referred to as SEQ ID NO: 14 (KKRN-VEEELHSLRKNYNIINEEIEEIT) or an ortholog sequence thereof from *P. vivax, P. berghei, P. knowlesi, P. chabaudi, P. yoelii*.

[0135] An immunogenic or vaccine composition of the invention can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder.

[0136] An immunogenic or vaccine composition of the invention may be in the form of a kit-of-part composition. For example, the immunogenic or vaccine composition of the invention may contain said at least one polypeptide of the invention and at least one other component, such as at least one adjuvant and/or at least one anti-*Plasmodium* immunogenic compound other than a polypeptide of the invention, in at least two distinct containers, preferably in at least two distinct vials, or in vials that keep said at least polypeptide of the invention separate from the other compounds of the composition until medical use is made of the composition. More preferably, an immunogenic or vaccine composition of the invention is a kit-of-part composition containing said at least one polypeptide of the invention and at least one adjuvant in separate containers, so that these two ingredients are kept separate until medical use thereof.

[0137] Preferably, said at least one polypeptide of the invention is contained in the immunogenic or vaccine composition of the invention under the form of a powder, more specifically under lyophilized form.

[0138] The immunogenic or vaccine composition of the invention may comprise one or several doses of said at least one polypeptide of the invention, the quantity of one dose being determined and/or adjusted by the physician taking due account of the patient's health, notably of the state of the patient's immunity system, and taking due account of the patient's medical features, such as age and weight. Illustrative doses are doses of 8 to 100 μg per adult person, preferably of 9 to 60 μg per adult person, e.g., of 10, 25 or 50 μg per adult person.

When formulated as a multidose composition, the immunogenic or vaccine composition of the invention may for example comprise said at least one polypeptide of the invention in a quantity corresponding to 2 to 20 individual doses, e.g., to 3 to 15 individual doses, said individual doses being either mixed together in the same container or vial or contained in individual containers or vials.

[0139] The administration schedule is to be determined by the physician depending on the patient's health, the stage of the Plasmodium infection, the patient's age, the patient's weight, and of the dose to be or that has already be administered. Typically, two or three doses at monthly interval are expected to be efficient in the treatment, more specifically the palliative and/or curative treatment of the disease.

[0140] The administration mode will be selected by the physician, and preferably is an administration by injection, more specifically an intramuscular injection.

[0141] The immunogenic or vaccine composition of the invention may further comprise at least one pharmaceutically and/or physiologically acceptable vehicle (diluent, excipient, additive, pH adjuster, emulsifier or dispersing agent, preservative, surfactant, gelling agent, as well as buffering and other stabilizing and solubilizing agent, etc.).

[0142] Appropriate pharmaceutically acceptable vehicles and formulations include all known pharmaceutically acceptable vehicles and formulations, such as those described in "Remington: The Science and Practice of Pharmacy", 20th edition, Mack Publishing Co.; and "Pharmaceutical Dosage Forms and Drug Delivery Systems", Ansel, Popovich and Allen Jr., Lippincott Williams and Wilkins.

In general, the nature of the vehicle will depend on the particular mode of administration being employed. For instance,

parenteral formulations usually comprise, in addition to the one or more contrast agents, injectable fluids that include pharmaceutically and physiologically acceptable fluids, including water, physiological saline, balanced salt solutions, buffers, aqueous dextrose, glycerol, ethanol, sesame oil, combinations thereof, or the like as a vehicle. The medium also may contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. The carrier and composition can be sterile, and the formulation suits the mode of administration.

[0143] For solid compositions (e.g., powder, pill, tablet, or capsule forms), conventional nontoxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, sodium saccharine, cellulose, magnesium carbonate, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

[0144] The composition can be formulated with traditional binders and carriers, such as triglycerides.

[0145] Preferably, an immunogenic or vaccine composition of the invention is kit-of-part composition comprising at least one, preferably several vials of a polypeptide of the invention under lyophilized form, wherein said polypeptide of the invention is intended to be dissolved in at least one adjuvant prior to administration thereof.

[0146] The invention also relates to a kit, which comprises at least one immunogenic or vaccine composition of the invention, and optionally a leaflet containing appropriate instructions for the use thereof as an immunogenic or vaccine composition, more particularly for the treatment of a Plasmodium-related disease, more specifically of malaria.

[0147] In view of what precedes, the present application also relates to methods for the treatment of an individual in need thereof, by active and/or passive immunotherapy, i.e., by at least one administration of at least one polypeptide of the invention, or of a nucleic acid coding therefore, preferably by injection, and/or by at least one administration of at least one antibody of the invention.

[0148] Also part of the present invention are **nucleic acids** (DNAs or RNAs) coding for a polypeptide of the invention, according to the universal genetic code and taking due account of the degeneracy of this genetic code, as well as oligonucleotide primers, which specifically amplify a sequence coding for a polypeptide of the invention (or a fragment thereof which is specific of such a sequence), preferably under stringent conditions, as well as oligonucleotide probes, which specifically hybridize to a polypeptide of the invention, preferably under stringent conditions.

[0149] Nucleic acids coding for a polypeptide of the invention advantageously comprise:

i) the sequence of SEQ ID NO: 5 (fragment 1-844 of the sequence of SEQ ID NO: 1);
ii) the sequence of a fragment of said sequence of SEQ ID NO: 5, said fragment having retained the sequence (SEQ ID NO: 9) extending from position 223 to position 326 of said sequence of SEQ ID NO: 5; or
iii) a conservative variant thereof, which derives from said sequence of SEQ ID NO: 5 of i) or from said fragment of SEQ ID NO: 5 of ii) by at least one conservative amino acid substitution and/or at least one conservative internal amino acid deletion, said conservative variant having retained the property of coding for a polypeptide, which has one, or at least one, preferably at least two, more preferably at least three, even more preferably at least four, still even more preferably at least five, yet still even more preferably at least six, most preferably at least seven, still most preferably at least eight, yet still most preferably all of the a)-i) properties, or one of the preferred combinations of the properties a) to i) as above-described.

[0150] The invention more particularly relates to a nucleic, the sequence of which is:

i) the sequence of a fragment of the sequence of SEQ ID NO: 5, said fragment sequence having retained the sequence (SEQ ID NO: 9) extending from position 223 to position 326 of said sequence of SEQ ID NO: 5; such as the sequence of SEQ ID NO: 15, 19, 9; or
ii) the sequence of a fragment of the sequence of SEQ ID No: 7, said fragment sequence having retained the sequence (SEQ ID NO: 11) extending from position 223 to position 326 of said sequence of SEQ ID NO: 7; such as the sequence of SEQ ID NO: 17, 21, 11;or
iii) a conservative variant sequence, which derives from said fragment sequences of i) or ii) by at least one conservative amino acid substitution and/or at least one conservative internal amino acid deletion, said conservative variant having retained the property of coding for a polypeptide, which has one, or at least one, preferably at least two, more preferably at least three, even more preferably at least four, still even more preferably at least five, yet still even more preferably at least six, most preferably at least seven, still most preferably at least eight, yet still most preferably all of the a)-i) properties, or one of the preferred combinations of the properties a) to i) as above-described.

[0151] A **primer** must be sufficiently long to prime the synthesis of the desired extension product. The exact length of the primer will depend upon many factors, including temperature, source of primer and use of the method.
A primer of the invention advantageously consist of 14-30 nucleotides, preferably of 15-29, more preferably of 16-28,

most preferably of 17-25 nucleotides, the sequences of which are suitable for use as forward or reverse primer, in the amplification of at least one nucleic acid encoding a polypeptide of the invention.

By "consisting of 14-30 nucleotides", it is meant "consisting of 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides".

A primer pair of the invention is a pair of two primers, which anneals to *Plasmodium* nucleic acid at such positions that the primer pair enables the specific amplification of an amplicon, said amplicon consisting of, or essentially consisting of, a nucleic acid coding for a polypeptide of the invention, or of a fragment of such a nucleic acid that is sufficiently specific to ascertain that the amplicon was contained in a nucleic acid coding for a polypeptide of the invention. By an "amplicon essentially consisting of", it is herein meant that the sequence that is amplified from the target *Plasmodium* nucleic acids consists of the indicated sequence, but that the amplicon sequence may further contain detection-related arm(s), such as beacon or scorpion arm(s), at its 5' and/or 3' ends.

The application also relates to a primer or a primer pair of the invention, for use in the detection of *Plasmodium* species, more specifically of *Plasmodium* falciparum strain(s), or for use in the diagnosis of a Plasmodium-related disease, such as malaria.

[0152] A **probe** must be sufficiently long to specifically hybridize to a polypeptide of the invention, preferably under stringent conditions.

[0153] A probe of the invention advantageously consists of 20-200 nucleotides, preferably of 25-100 nucleotides.

[0154] The application also relates to a probe of the invention, for use in the detection of *Plasmodium* species, more specifically of *Plasmodium* falciparum strain(s), or for use in the diagnosis of a Plasmodium-related disease, such as malaria.

[0155] The invention also relates to a PCR system, comprising at least one primer or primer pair of the invention and at least one probe of the invention.

[0156] The application also relates to a PCR system of the invention, for use in the detection of *Plasmodium* species, more specifically of *Plasmodium* falciparum strain(s), or for use in the diagnosis of a *Plasmodium*-related disease, such as malaria.

[0157] The invention also pertains to a recombinant **cloning and/or expression vector,** comprising at least one nucleic acid of the invention. In a vector of the invention, said at least one nucleic acid can be under the control of a promoter and regulatory elements homologous or heterologous vis-à-vis a host cell, for expression in said host cell.

[0158] An expression vector as described in the above paragraph can advantageously be used for the preparation of a medicament for genetic immunisation against *Plasmodium* species, more specifically against *P. falciparum.*

A recombinant or genetically engineered host cell, for example a bacterium, a yeast, an insect cell, or a mammalian cell, which is transformed by an expression vector as described above, is also part of the present invention.

[0159] The invention also pertains to **a nucleic acid vaccine** (e.g. polynucleotide vaccine) comprising at least one nucleic acid, vector or host cell of the invention.

[0160] Several aspects and advantages of the present invention are illustrated in the following figures and experimental data.

[0161] The following **definitions** apply throughout the text of the present application.

[0162] In the context of the present invention, 'amino acid' or **'amino acid residue'** means any amino acid residue known to those skilled in the art (see e.g.: Sewald *et al.*, 2002; IUPAC nomenclature under http://www.chem.qmul.ac.uk/iupac/AminoAcid/).

[0163] This encompasses naturally occurring amino acids (including for instance, using the three-letter code, Ala, bAla, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val), as well as rare and/or synthetic amino acids and derivatives thereof (including for instance Aad, Abu, Acp, Ahe, Aib, Apm, Dbu, Des, Dpm, Hyl, MeLys, MeVal, Nva, HAO, NCap, Abu, Aib, MeXaa and the like (see e.g.: (Müller *et al.,* 1993; Aurora *et al.,* 1998; Obrecht *et al.,* 1999; Maison *et al.*, 2001; Formaggio *et al.*, 2003;Nowick *et al.*, 2003).

[0164] Said amino acid residue or derivative thereof can be any isomer thereof, especially any chiral isomer, e.g., the L- or D- isoform.

[0165] By amino acid derivative, we hereby mean any amino acid derivative as known in the art (see e.g.: Sewald *et al.,* 2002; IUPAC nomenclature under http://www.chem.qmul.ac. uk/iupac/AminoAcid/).

[0166] For instance, amino acid derivatives include residues derivable from natural amino acids bearing additional side chains, e.g. alkyl side chains, and/or heteroatom substitutions. Further examples of amino acid derivatives comprise amino acid bearing chemical modifications such the one fund in mimetic peptides or peptidomimetics, which are compounds containing non-peptidic structural elements that are capable of mimicking or antagonizing the biological action (s) of a natural parent peptide. A peptidomimetic usually does no longer have classical peptide characteristics such as enzymatically scissille peptidic bonds.

[0167] Preferably, said amino acid belongs to the group of the non-essential amino acids. Preferred non-essential amino acids are glycine, alanine, proline, serine, cysteine, tyrosine, asparagines, glutamine, aspartic acid, glutamic acid, arginine, histidine.

Appropriate amino acids may be accurately selected by selecting those amino acids which are in lower amounts in the patient into which the drug is to be administered. Dosage and administration regimen can be determined as a function of the patient's level in said amino acid. Preferred dosage and administration regimen are those which intend to increase the patient's amino acid level up to the normal standard level.

[0168] **Unfolded or unstructured regions** can de determined by identification of amino acid stretches, which have a highly hydrophilic amino acid sequence. Such stretches have no hydrophobic core.

[0169] **Hydropathy index** of standard amino acids is as follows (Kyte and Doolittle 1982. J. Mol. Biol. 157 (1): 105-132):

Amino acids sorted by increasing hydropathy index																			
R	**K**	**N**	**D**	**Q**	**E**	**H**	**P**	**Y**	**W**	**S**	**T**	**G**	**A**	**M**	**C**	**F**	**L**	**V**	**I**
-4.5	**-3.9**	**-3.5**	**-3.5**	**-3.5**	**-3.5**	**-3.2**	**-1.6**	**-1.3**	**-0.9**	**-0.8**	**-0.7**	**-0.4**	**1.8**	**1.9**	**2.5**	**2.8**	**3.8**	**4.2**	**4.5**

[0170] The larger the value of the hydropathy index is, the more hydrophobic the amino acid. The most hydrophobic amino acids are isoleucine (4.5) and valine (4.2). The most hydrophilic ones are arginine (-4.5) and lysine (-3.9). Hydrophobic amino acid residues notably include P, Y, W, S, T, G, A, M, C, F, L, V, I.

[0171] The person of average skill in the art uses a **computer tool** to predict unstructured or unfolded regions, such as the DisEMBL software (DisEMBL-1.4), which is available on http://dis.embl.de/html/help.html. The DisEMBL software provides three predictors, namely the Loops/coils predictor, the Hot-loops predictor and the REMARK-465 predictor

[0172] The Loops/coils predictor uses the Definition of Secondary Structure of Proteins (DSSP) program. Executables of the DSSP program are available from hftp://www.embl-heidelberg.de/dssp/ or from ftp.embl-heidelberg.de. With the Loops/coils predictor, residues are assigned as belonging to one of several secondary structure types. Residues as alpha -helix ('H'), 3_10-helix ('G') or beta-strand ('E') are considered to ordered residues, and all other states ('T', 'S', 'B', 'I', ' ') are considered to be loops (also known as coils). Loops/coils are not necessarily disordered; however, protein disorder is only found within loops. It follows that one can use loop assignments as a necessary but not sufficient requirement for disorder. The Hot-loops predictor constitutes a subset of the Loops/coils predictor, namely those loops with a high degree of mobility as determined from C-alpha temperature (B-)factors. It follows that highly dynamic loops should be considered protein disorder. Missing coordinates in X-Ray structure as defined by REMARK-465 entries in PDB. Non assigned electron densities most often reflect intrinsic disorder, and are used early on in disorder prediction. Preferably, the Hot-Loops or the REMARK465 predictor of the DisEMBL software is being used.

[0173] Most preferably, the identification of an unstructured or unfolded region of *Plasmodium* protein is made with the Hot-Loops predictor of the DisEMBL-1.4 software, the six parameters of the software being left at their default settings.

[0174] The term "**sequence identity"** has its ordinary meaning in the field. The terms "identical" or percent "identity" in the context of two or more polypeptide sequences, refer to two or more sequences that are the same, or have a specified percentage of amino acid residues that are the same (i.e., at least 70% identity, preferably at least 75%, 80%, 85%, 90%, or 95% identity over a specified region), when compared and aligned for maximum correspondence. A preferred example of algorithm that is suitable for determining percent sequence identity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402 (1977) and Altschul et al., J. Mol. Biol. 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used to determine percent sequence identity for the polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.,* supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0175] The person of average skill in the art usually consider that the following eight groups each contain amino acids that are **conservative substitutions** for one another:

1) Alanine (A), Glycine (G);

2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M);
(see, e.g., Creighton, Proteins (1984).

[0176] The term **"treatment"** or "treating" herein encompasses curative treatment, preventive treatment as well as palliative treatment, more specifically palliative treatment and curative treatment.

[0177] The term **"palliative treatment**" herein reflects that the fact that the treatment concerned focuses on the blood stages of the *Plasmodium* infection, and therefore that it does not aim at preventing infection or at destroying the parasites at the pre-erythrocytic stage, but at:

- reducing the morbidity and/or mortality, and/or at
- accelerating the acquisition of natural immunity against the parasite and/or at
  maintaining the natural immunity at a level appropriate that is appropriate to the patient's health.

[0178] The term "carrier" or **"carrier molecule"** means an immunogenic molecule containing antigenic determinants recognized by T cells. A carrier molecule can be a protein or can be a lipid. A carrier protein is conjugated to a polypeptide to render the polypeptide immunogenic, or to further increase its immunogenicity. Carrier proteins include keyhole limpet hemocyanin, horseshoe crab hemocyanin, and bovine serum albumin.

[0179] The term **"adjuvant"** is as defined by EMEA, i.e., a component that potentiates the immune responses to an antigen and/or modulates it towards the desired immune responses. These adjuvants include for instance:

- Freund's adjuvant, either complete or incomplete; Titermax® gold adjuvant; alum; LPS such as bacterial LPS; gamma-linolenic acid (GLA), such as GLA-57, GLA-27, GLA-58, GLA-59, GLA-60; Montanide® ISA 720;
- Mineral salts, e.g., aluminium hydroxide and aluminium or calcium phosphate gels;
- Oil emulsions and surfactant based formulations, e.g., MF59 (microfluidised detergent stabilised oil-in-water emulsion), QS21 (purified saponin), AS02 [SBAS2] (oil-in-water emulsion + MPL + QS-21), Montanide ISA-51 and ISA-720 (stabilised water-in-oil emulsion);
- Particulate adjuvants, e.g., virosomes (unilamellar liposomal vehicles incorporating influenza haemagglutinin), AS04 ([SBAS4] Al salt with MPL), ISCOMS (structured complex of saponins and lipids), polylactide co-glycolide (PLG);
- Microbial derivatives (natural and synthetic), e.g., monophosphoryl lipid A (MPL), Detox (MPL + M. Phlei cell wall skeleton), AGP [RC-529] (synthetic acylated monosaccharide), DC_Chol (lipoidal immunostimulators able to self organise into liposomes), OM-174 (lipid A derivative), CpG motifs (synthetic oligonucleotides containing immunostimulatory CpG motifs), modified LT and CT (genetically modified bacterial toxins to provide non-toxic adjuvant effects);
- Endogenous human immunomodulators, e.g., hGM-CSF or hIL-12 (cytokines that can be administered either as protein or plasmid encoded), Immudaptin (C3d tandem array);
- Inert vehicles, such as gold particles.

[0180] Other novel types of adjuvants not listed above may be under development and are also encompassed by the present application.

[0181] "**Stringent hybridization conditions**" are defined herein as conditions that allow specific hybridization of two nucleic acid especially two DNA molecules at about 65°C, for example in a solution of 6X SSC, 0.5% SDS, 5X Denhardt's solution and 100 μg/ml of denatured non specific DNA or any solution with an equivalent ionic strength, and after a washing step carried out at 65°C, for example in a solution of at most 0.2X SSC and 0.1% SDS or any solution with an equivalent ionic strength. However, the stringency of the conditions can be adapted by the skilled person as a function of the size of the sequence to be hybridized, its GC nucleotide content, and any other parameter, for example following protocols described by Sambrook et al, 2001 (Molecular Cloning: A Laboratory Manual, 3rd Edition, Laboratory Press, Cold Spring Harbor, New York).

[0182] The term "**comprising**", which is synonymous with "including" or "containing", is open-ended, and does not exclude additional, unrecited element(s), ingredient(s) or method step(s), whereas the term "consisting of" is a closed term, which excludes any additional element, step, or ingredient which is not explicitly recited.
The term "essentially consisting of" is a partially open term, which does not exclude additional, unrecited element(s),

step(s), or ingredient(s), as long as these additional element(s), step(s) or ingredient(s) do not materially affect the basic and novel properties of the invention.

[0183] The term "comprising" (or "comprise(s)") hence includes the term "consisting of" ("consist(s) of"), as well as the term "essentially consisting of" ("essentially consist(s) of"). Accordingly, the term "comprising" (or "comprise(s)") is, in the present application, meant as more particularly encompassing the term "consisting of" ("consist(s) of"), and the term "essentially consisting of" ("essentially consist(s) of").

[0184] Each of the relevant disclosures of all references cited herein is specifically incorporated by reference.

[0185] The present invention is illustrated by the following examples, which are given for illustrative purposes only.

EXAMPLES

Example 1 *: In vitro* blood stage killing of *P. falciparum* by antibodies to the gene products, by the ADCI mechanism

2.A. Materials and methods : the ADCI assay

2.A.1. Introduction

[0186] The Antibody Dependent Cellular Inhibition (ADCI) assay is designed to assess the capability of antibodies to inhibit the in vitro growth of *Plasmodium,* more particularly of *Plasmodium falciparum* in the presence of monocytes. Studies have shown that antibodies that proved protective against *P. falciparum* blood stages by passive transfer in humans are unable to inhibit the parasite in vitro unless they are able to cooperate with blood monocytes. It has also been shown that antibodies that were not protective in vivo had no effect on *P. falciparum* growth in the ADCI assay. The ADCI is therefore an in vitro assay the results of which reflect the protective effect of anti-malarial antibodies observed under in vivo conditions in humans.

[0187] The antibodies able to cooperate with monocytes should be obviously cytophilic: IgG1 and IgG3 isotypes are efficient in ADCI while IgG2, IgG4 and IgM are not efficient. This is consistent with the findings that in sera from protected individuals, cytophilic anti-*P.falciparum* antibodies are predominant, while in non-protected patients the antibodies produced against the parasite are mostly non-cytophilic.

The results suggest that ADCI likely involves the following succession of events: at the time of schizonts rupture, the contact between some merozoite surface component and cytophilic antibodies bound to monocytes via their Fc fragment triggers the release of soluble mediators which diffuse in the culture medium and block the division of surrounding intra-erythrocytic parasites.

[0188] The major steps of the ADCI protocol are:

(i). Serum IgG preparation using ion exchange chromatography
(ii). Monocyte isolation from a healthy blood donor
(iii). Preparation of *P.falciparum* parasites including synchronization and schizont enrichment.
(iv). Parasite culture, for 96 hrs, in the presence of antibodies and monocytes.
(v). Inhibition effect assessed by microscopic observation and parasite counting.

[0189] The ADCI assay is also described in Hasnaa Bouharoun-Tayoun et al. 1995 J. Exp. Med. 182: 409-418.

2.A.2. Materials

IgG Preparation

[0190]

1. Tris buffer: 0.025 *M* Tris-HCl, 0.035 *M* NaCl, pH 8.8.
2. Phosphate Buffer Saline (PBS), pH 7.4.
3. GF-05-Trisacryl filtration column (IBF, Biothecnics, Villeneuve La Garenne, France).
4. DEAE-Trisacryl ion exchange chromatography column (IBF).
5. G25 Filtration column.
6. Amicon filters and tubes for protein concentration (Mol. Wt. cut off: 50,000 Da).
7. Sterile Millex filters, 0.22 μm pore size (Millipore Continental Water Systems, Bedford MA).
8. Spectrophotometer equipped with Ultra Violet lamp.

Monocyte Preparation

[0191]

1. Heparinized blood collected from a healthy donor, 20-40 mL volume.
2. Ficoll-Hypaque density gradient (Pharmacia LKB Uppsala, Sweden).
3. Hank's solution supplemented with $NaHCO_3$, pH 7.0.
4. RPMI 1640 culture medium supplemented with 35 mM Hepes and 23 mM $NaHCO_3$; prepare with mineral water; store at 4°C.
5. Reagents for non-specific esterase (NSE) staining: fixing solution, nitrite, dye, buffer and substrate
6. 96-well sterile plastic plates (TPP, Switzerland).
7. Refrigerated centrifuge.
8. CO_2 incubator.
9. Inverted microscope.

Parasite Preparation

[0192]

1. RPMI 1640 culture medium (see above).
2. 10% Albumax stock solution; store at 4°C for up to 1 month.
3. 5% Sorbitol for parasite synchronization.
4. Plasmagel for schizont enrichment.
5. Reagents for fixing and staining of thin smears: methanol, eosine, methylene blue.

2.A.3. Methods

IgG preparation

[0193] IgGs are extracted from human sera (see Note 1) as follows:

1. Dilute the serum at a ratio of 1 to 3 in Tris buffer.
2. Filter the diluted serum through a GF-05 Trisacryl gel filtration column previously equilibrated in the Tris buffer. Ensure that the ratio of serum to filtration gel is 1 volume of undiluted serum to 4 volumes of GF-05 gel.
3. Pool the protein-containing fractions
4. Load over a DEAE-Trisacryl ion exchange chromatography column previously equilibrated with Tris buffer. Ensure that the ratio of serum to filtration gel is 1 volume of undiluted serum to 4 volumes of DEAE gel.
5. Collect fractions of 1 mL volume.
6. Measure the optical density (OD) of each fraction using a 280 nm filter.
7. Calculate the IgG concentration as follows: $$\text{IgG concentration (mg/mL)} = \frac{\text{OD 280 nm}}{1.4}$$
8. Pool the fractions containing IgGs.
9. Concentrate the IgG solution using Amicon filters. Amicon filters are first soaked in distilled water for 1 hour and than adapted to special tubes in which the IgG solution is added.
10. Centrifuge the tubes at 876g for 2 hr at 4°C. This usually leads to a 25-fold concentration.
11. Perform a final step of gel filtration using a G25 column previously equilibrated in RPMI culture medium.
12. Collect the IgG fractions in RPMI.
13. Measure the optical density (OD) of each fraction using a 280 nm filter.
14. Calculate the IgG concentration.
15. Pool the fractions containing IgGs.
16. Sterilize the IgG fractions by filtration through 0.22 μm pore size filters.
17. Store the sterile IgG solution at 4°C for up to 1 month (or add Albumax for longer storage- but not recommended-).

Monocyte Preparation

[0194] The procedure for monocyte preparation is based on that described by Boyum (Scand. J. Clin. Lab. Invest.

1968, 21, 77-89) and includes the following steps:

1. Dilute the heparinized blood 3-fold in Hank's solution.
2. Carefully layer two volumes of diluted blood onto 1 volume of Ficoll-Hypaque (maximum volume of 20 mL of diluted blood per tube).
3. Centrifuge at 560 g for 20 min at 20°C.
4. Remove the mononuclear cell layer at the Ficoll/plasma interface.
5. Add 45 mL of Hank's solution to the mononuclear cell suspension.
6. Centrifuge at 1000 g for 15 min at 20°C.
7. Carefully resuspend the pelleted cells in 45 mL of Hank's solution.
8. Centrifuge again at 1000 g for 15 min at 20°C. Repeat this washing step twice more.
9. Finally, centrifuge at 180 g for 6 min at 20°C, to remove any platelets that remains in the supernatant.
10. Resuspend the mononoclear cells in 2 mL of RPMI.
11. Calculate the mononuclear cell concentration (i.e. lymphocytes plus monocytes) in the cell suspension: dilute a 20 μL aliquot of the cell suspension 3-fold in RPMI and count cell numbers using a hemocytometer (Malassez type for example).
12. Determine the number of monocytes using the Non Specific Esterase (NSE) staining technique:

    (i). In microtube A, add 40 μL of mononuclear cell suspension to 40 μL of fixing solution.
    (ii). In microtube B, mix the NSE staining reagents in the following order: 60 μL of nitrite, 60 μL of dye, 180 μL of buffer, and 30 μL of substrate
    (iii). Add the mixture in microtube B to the cells in microtube A.
    (iv). Take a 20 μL sample of the stained cells and measure the proportion of monocytes : lymphocytes: monocytes will be colored in brown whereas the lymphocytes will be uncolored. Usually the proportion of monocytes is 10-20% of the total mononuclear cells.

13. Adjust the cell suspension to a concentration of 2 x 10^5 monocytes per 100 μL, with RPMI.
14. Aliquot the cell suspension in a 96-well plate at 100 μL/well.
15. Incubate for 90 min at 37°C, 5% CO_2. During this incubation, monocytes will adhere to the plastic.
16. Remove the non-adherent cells and wash the monocytes by adding, and thoroughly removing, 200 μL of RPMI in each well.
17. Repeat this washing procedure 3 times in order to remove all the non-adherent cells.
18. At least 95% of the recovered cells will be monocytes. Control for the cell appearance and the relative homogeneity of cell distribution in the different wells by observation using an inverted microscope (see Notes 2, 3, and 4)

Parasite Preparation

P. falciparum strains are cultivated in RPMI 1640 supplemented with 0.5% Albumax.

[0195] Parasites are synchronized by Sorbitol treatments as follows:

1. Dilute the sorbitol stock to 5% in mineral water.
2. Centrifuge the asynchronous parasite culture suspension at 1200 rpm for 10 min at 20°C.
3. Resuspend the pellet in the 5% sorbitol solution. This will lead to the selective lysis of schizont infected RBC without any effect on the rings and young trophozoites.

[0196] When required, schizonts are enriched by flotation on plasmagel as follows:

1. Centrifuge cultures containing asynchronous parasites at 250 g for 10 min at 20°C
2. Resuspend the pellet at a final concentration of 20% red blood cells (RBC), 30% RPMI, 50% plasmagel.
3. Incubate at 37°C for 30 min. Schizont-infected RBC will remain in the supernatant, whereas young trophozoite-infected and uninfected RBC will sediment.
4. Collect carefully the supernatant, by centrifugation at 250 g for 10 min at 20°C.
5. Prepare a thin smear from the pelleted cells, stain, and determine the parasitemia by microscopic examination.
6. Usually, using this method, synchronous schizont infected RBC are recovered at ~ 70 % parasitemia.

[0197] For the ADCI assay, synchronized early schizont parasites are used. Usually the parasitemia is 0.5-1.0% and the hematocrit 4%.

The ADCI Assay

[0198]

1. After the last washing step, add in each monocyte containing well:

   (i). 40 μL of RPMI supplemented with 0.5% Albumax (culture medium).
   (ii). 10 μL of the antibody solution to be tested. Usually the IgGs are used at 10% of their original concentration in the serum (~ 20 mg/mL for adults from hyperendemic areas, and ~ 12 mg/mL for children from endemic area and primary attack patients). (see Note 5).
   (iii). 50 μL of parasite culture, at 0.5% parasitemia and 4% hematocrit.

2. Control wells consist of the following elements:

   (i). Monocytes (MN) and parasites with normal IgG (N IgG) prepared from the serum of a donor with no history of malaria.
   (ii). Parasite culture with IgG to be tested without MN.

3. Maintain the culture at 37°C for 96 hrs in a candle-jar (or a low O_2, 5% CO_2 incubator).
4. Add 50 μL of culture medium to each well after 48 and 72 hrs.
5. Remove the supernatant after 96 hrs. Prepare thin smears from each well, stain, and determine the parasitemia by microscopic examination. In order to ensure a relative precision in the parasite counting, a minimum of 50,000 red blood cells (RBC) should be counted and the percentage of infected RBC calculated (see Notes 6 and 7).
6. Calculate the specific Growth Inhibitory Index (SGI), taking into account the possible inhibition induced by monocytes or antibodies alone:
SGI = 100 X (1- [Percent parasitemia with MN and Abs / Percent parasitemia with Abs] / [Percent parasitemia with MN+ N IgG / Percent parasitemia with N IgG])

2.A.4. notes

[0199]

1. IgG preparation from sera to be tested is an essential step because a non-antibody dependent inhibition of parasite growth has frequently been observed when unfractionated sera were used, probably due to oxidized lipids.
2. Monocyte (MN) function in ADCI is dependent upon several factors such as water used to prepare RPMI 1640. Highly purified water, such as Millipore water, although adequate for parasite culturing, leads to a poor yield in the number of MN recovered after adherence to the plastic wells. On the other hand, water which contains traces of minerals, such as commercially available Volvic water, or glass-distilled water, provide consistently a good monocyte function.
3. Improved monocyte adherence can be obtained by coating the culture wells with fibronectin i.e. coating with autologous plasma from the MN donor, followed by washing with RPMI 1640, prior to incubation with mononuclear cells.
4. MN from subjects with a viral infection (e.g. influenza) are frequently able to induce a non IgG dependent inhibition of parasite growth. This non-specific inhibition effect could prevent the observation of the IgG-dependent inhibition in ADCI. Therefore, MN donors suspected of having a viral infection, or who have had fever in the past 8 days, should be avoided. The results from ADCI are not reliable when the direct effect of MN alone is greater than 50% inhibition. The preparation of MN in medium containing heterologous serum, such as FCS, results in the differenciation of MN, their progressive transformation into macrophages which have lost their ADCI promoting effect.
5. If required, murine IgG can be tested in ADCI with Human MN. The IgG2a isotype is able to bind to the human Fc γ receptor II present on monocytes shown to be involved in the ADCI mechanism.
6. A possible variation of the ADCI assay is the assessment of a competition effect between protective cytophilic antibodies (adults from hyperendemic area) directed to the merozoite surface antigens, and non-protective antibodies (children from endemic area and primary attack patients) which recognize the same antigens but are not able to trigger the monocyte activation because they do not bind to Fc gamma receptors. Therefore non-cytophilc Ig directed to the "critical" antigens may block the ADCI effect of protective antibodies. Each IgG fraction should be used at 10% of its original concentration in the serum.
7. The ADCI assay protocol can be modified and performed as a two-step ADCI with short-term activation of monocytes according to the following procedure:

(i). Incubate MN for 12-18 hrs with test Ig and synchronous mature schizonts infected RBC, at 5-10% parasitemia. During this first culture time, infected RBC rupture occurs and merozoites are released.
(ii). Collect supernatants from each well and centrifuge them at 700 g.
(iii). Distribute the supernatants in a 96-well plate, at 100 μL/well
(iv). Add to each well 100μL of *P.falciparum* asynchronous culture containing fresh medium, at 0.5-1% parasitemia, 5% hematocrit (particular care is taken to reduce to a minimum the leucocyte contamination of the RBC preparation used for this second culture).
(v). At 36 hr of culture, add 1 mCi of ^{3}H hypoxanthine to each well.
(vi). At 48 hr of culture, harvest cells and estimate ^{3}H uptake by counting in a liquid scintillation counter.

Example 2:

- Structure

[0200] The unfolded or unstructured 3D-arrangement of the P27A polypeptide is illustrated by Figure 5.

- Life cycle stage of expression

[0201] The protein **PFF0165c/ MAL6P1.37 (*Pf*27)** investigated by Villard *et al* (Plos One 2: e645) is predicted to be expressed during the blood stage of *Plasmodium falciparum* infection (PlasmoDB). Indeed, human affinity-purified antibodies and murine antibodies induced by immunization against **P27** and **P27A** specifically stain *Plasmodium falciparum*-infected erythrocytes, but not sporozoites (Fig. 2).
[0202] In Western blots of lysates of infected erythrocytes (Fig. 3), these antibodies also reacted specifically with a protein of the expected molecular weight (about 130kD), as well as with a small number of bands of lower molecular weight, which may represent degradation products.

- **Accessibility**

[0203] Immunofluorescence staining of *Plasmodium falciparum*-infected erythrocytes by human affinity-purified antibodies against **P27** and **P27A,** and also antibodies induced in mice against **P27A** and **P27,** is localised to cytoplasm and the periphery of infected erythrocytes. However it is not co-localized with the Maurer's cleft associated histidine rich protein (MAHRP) (Fig. 2).

- Protein function

[0204] The biological and biochemical functions of the malaria protein **PFF0165c (Pf 27)** are still unknown and have not been investigated in the course of our preclinical studies.

***- In vivo* protection**

[0205] Detailed immuno-epidemiological studies show that cytophilic human antibodies strongly correlate with acquired clinical protection from malaria.

***- In vitro* inhibition**

[0206] Human affinity-purified antibodies specific for **P27** and **P27A,** as well as murine antibodies induced by immunization were extremely potent in the ADCI assays (i.e., as active as purified immunoglobulin pool prepared from sera of adults living in malaria-endemic areas) (Villard *et al.,* Plos One 2: e645).
[0207] Results are normalized with respect to the Pool of Immune African Globulins (PIAG; cf. Hasnaa Bouharoun-Tayoun et al. 1990 J. Exp. Med. 172: 1633-1641), which is used as a reference showing 100% activity.
[0208] The results are as follows:

- with immunopurified human antibodies (final concentration of 15 μg/mL):

  for P27, the SGI is of 106%, and
  for P27A, the SGI is of 80-85%;

- with the sera from immunized mice:

[0209]

for P27, with ICR mice, final titer of Indirect Fluorescent Antibody (IFA) in the well at 1/40, SGI = 100%;
for P27A, with C3H mice, final titer of Indirect Fluorescent Antibody (IFA) in the well at 1/20, SGI = 92%;
for P27+P27A, with C3H mice, final titer of Indirect Fluorescent Antibody (IFA) in the well at 1/40, SGI = 120%.

- Diversity (e.g. sequence and antigen diversity (minimal sequence variation))

[0210] Genetic diversity of **P27** and **P27A** was assessed by using *in vitro* cultured strains and field samples of Pf from two malaria endemic areas. PCR primers were designed for amplifying the two regions within ***Pf*27** corresponding to **P27** and **P27A.** Nucleotides were aligned to screen for polymorphism within the sequences corresponding to the two peptides.

P27 forward primer:

[0211]

TCTCTTCTACATACGCTTTATTCA (SEQ ID NO: 55)
P27 reverse primer:
GATAATTCGTTTAATGAGGAGTCCA (SEQ ID NO: 56)

P27A forward primer:

[0212]

ACACTTTGCACAGTTCCTATCTTCTCTTCTA (SEQ ID NO: 57)
P27A reverse primer:
AGAAGGAGAAGAAGAAAATAAAGAGGATGAAG (SEQ ID NO: 58)

[0213] **P27-** No polymorphism whatsoever was observed in 46 Tanzanian blood samples, 17 samples from Papua New Guinea and 8 in vitro culture strains.

[0214] **P27A-** shows limited polymorphism with essentially a single SNP (E292G, see preceding Section) being distributed worldwide and reaching a high prevalence (6/19 samples from Papua New Guinea- frequency 0.31, 38/63 samples from Tanzania-frequency 0.6, and 4/11 isolates from PlasmoDB. This gives an overall frequency of 0.52). Two additional SNPs in **P27A** were each only observed in one isolate from PlasmoDB by microarray, a technique prone to false positives (Kidgell et al. PLoS Pathog. 2006, 2:e57).

[0215] Orthologs of ***Pf27*** are found in other species of *Plasmodium - P. vivax, P. knowlesi, P. chabaudi, P. yoelii* and *P. berghei* (PlasmoDB).

- Seroepidemiological data

[0216] Below are the antibody prevalence* and mean OD measured by ELISA of individual endemic-area sera against **P27** and **P27A** (Villard *et al.*, cited supra).

[0217] *percent of donors whose serum gave an OD value in ELISA exceeding mean OD + 3 standard deviations of negative controls (naïve European donors).

Table 2:

	Burkina Faso (N = 37)		Tanzania (N = 42)	
	% prevalence	Mean OD	% prevalence	Mean OD
Peptide 27	**54**	**0.265**	**69**	**0.237**
Peptide 27A	**76**	**0.673**	**76**	**0.688**

[0218] IgG1 and IgG3, known to be associated with antibody-dependent cellular inhibition and protection against

intracellular organisms, are the predominant IgG subclasses in the anti-peptide antibodies.

[0219] Further studies were performed among inhabitants of Dielmo, Senegal. The first study involved 45 individuals among whom 22 had no malaria attacks during 3-year of follow-up period and 23 had one or more malaria attacks during the same period. Both have the same age-distribution. These results were confirmed among 102 Ndiop inhabitants while also adjusting for age and occurrence of malaria attacks.

[0220] The prevalence, cytophilic IgG subclass and association with protection were studied against the 14 initial peptides/genes selected (Fig 4). Please see the prevalence values indicated below the diagram of Figure 4: the total proportion of individuals responding by IgG1 and IgG3, i.e., the most critical IgG subclasses, is higher for the polypeptide of the invention P27A (IgG1: 86.7%; IgG3 82.2%) than for the prior art peptide P27 (IgG1 6.7%; IgG3 95.6%). Indeed, the proportion of individuals responding by IgG1 is drastically higher for P27A (82.2%) than for P27 (6.7%). The peptides of Figure 4 are shown in Table 5 below.

Table 5 :

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
p14	27	MAL6P1.37 (PFF0165c)	**Predicted Protein Sequence of 1103aa (in underlined characters: fragment of the protein corresponding to peptide 27)** MSNKKRSKNENDESTSLPLENSELLIEYIHNLKSCLNVYRREIQEKNKYISIIKNDLSFH ECILTNVNVVWSVFNNDLLNLLCNNEQKEEGEEIIKQRNIGDEINEYNNLTKLQNDENIK NNNMIKEDLEDDANQNILMKSPYYNIENFLQVFLKYINKKKKKVKVKVKDEGKKEKIEDK KYEQDDEEENEEEEEEEEEEEGEEENKEDEEFFKTFVSFNLYHNNNEKNISYDKNLVKQE NDNKDEARGNDNMCGNYDIHNERGEMLDKGKSYSGDEKINTSDNAKSCSGDEKVITSDNG KSYDYVKNESEEQEEKENMLNNKKRSLECNPNEAKKICFSLEEKIGTVQSVKLKEYNELS KENIEKNKHDDNNICNYLSHNEGENVIEREDKLFNKLNNKNYRNEEEKKKNQINFDYLKK KIKNNQDVFEETIQKCFLINLKKTLNLINKIMYLKNVEFRKYNLDYIRKINYEKCFYYKN YIDIKKKISELQKDNESLKIQVDRLEKKKATLIYKLNNDNIRKHILDNNIKDYQNGIDNS KVSYFDEGENPYNRNNKNYRTDNKNSDDNNNNNNYYYNNYNSDDNYNSEDNEYNNGNYRF RNNYKKDSLNEDDVKKNPLKVCHKINSDSNIFVNFENIITKQNIIHSEPFRNLLKESNEL YITLKEKEKENIILKNEILKMENKKDEEYEHLLNNTIEDKKELTRSIKELEINMMTCNME KDKISNKVNTLEYEINVLKNIDKNQTMQLQQKENDILKMKLYIEKLKLSEKNLKDKIILL ENEKDKMLSGIHIKDNSFNEESKSEEGKIQLRDIQNDNDEKYDDEKKRFKELFIENQKLK EELNKKRNVEEELHSLRKNYNIINEEIEEITKEFEKKQEQVDEMILQIKNKELELLDKFN NKMNKAYVEEKLKELKNTYEEKMKHINNIYKKHDDFVNIYLNLFFQARKNAILSDSQREE QMNLFIKLKDKYDIIFQKKIELTDILKNVYDCNKKLIGHCQDLEKENSTLQNKLSNEIKN SKMLSKNLSKNSDDHLLIEENNELRRRLICSVCMENFRNYIIIKCGHIYCNNCIFNNLKT RNRKCPQCKVPFDKKDLQKIFLD **(SEQ ID NO: 27)** p27: KKRNVEEELHSLRKNYNIINEEIEEIT **(SEQ ID NO: 28)**
p27A	27A	MAL6P1.37 (PFF0165c)	**Predicted Protein Sequence of 1103aa (in underlined characters: fragment of the protein corresponding to peptide 27A)** MSNKKRSKNENDESTSLPLENSELLIEYIHNLKSCLNVYRREIQEKNKYISIIKNDLSFH ECILTNVNVVWSVFNNDLLNLLCNNEQKEEGEEIIKQRNIGDEINEYNNLTKLQNDENIK NNNMIKEDLEDDANQNILMKSPYYNIENFLQVFLKYINKKKKKVKVKVKDEGKKEKIEDK KYEQDDEEENEEEEEEEEEEEGEEENKEDEEFFKTFVSFNLYHNNNEKNISYDKNLVKQE NDNKDEARGNDNMCGNYDIHNERGEMLDKGKSYSGDEKINTSDNAKSCSGDEKVITSDNG KSYDYVKNESEEQEEKENMLNNKKRSLECNPNEAKKICFSLEEKIGTVQSVKLKEYNELS KENIEKNKHDDNNICNYLSHNEGENVIEREDKLFNKLNNKNYRNEEEKKKNQINFDYLKK KIKNNQDVFEETIQKCFLINLKKTLNLINKIMYLKNVEFRKYNLDYIRKINYEKCFYYKN YIDIKKKISELQKDNESLKIQVDRLEKKKATLIYKLNNDNIRKHILDNNIKDYQNGIDNS KVSYFDEGENPYNRNNKNYRTDNKNSDDNNNNNNYYYNNYNSDDNYNSEDNEYNNGNYRF RNNYKKDSLNEDDVKKNPLKVCHKINSDSNIFVNFENIITKQNIIHSEPFRNLLKESNEL YITLKEKEKENIILKNEILKMENKKDEEYEHLLNNTIEDKKELTRSIKELEINMMTCNME KDKISNKVNTLEYEINVLKNIDKNQTMQLQQKENDILKMKLYIEKLKLSEKNLKDKIILL ENEKDKMLSGIHIKDNSFNEESKSEEGKIQLRDIQNDNDEKYDDEKKRFKELFIENQKLK EELNKKRNVEEELHSLRKNYNIINEEIEEITKEFEKKQEQVDEMILQIKNKELELLDKFN NKMNKAYVEEKLKELKNTYEEKMKHINNIYKKHDDFVNIYLNLFFQARKNAILSDSQREE QMNLFIKLKDKYDIIFQKKIELTDILKNVYDCNKKLIGHCQDLEKENSTLQNKLSNEIKN SKMLSKNLSKNSDDHLLIEENNELRRRLICSVCMENFRNYIIIKCGHIYCNNCIFNNLKT RNRKCPQCKVPFDKKDLQKIFLD **(SEQ ID NO: 29)** p27A: HNNNEKNISYDKNLVKQENDNKDEARGNDNMCGNYDIHNERGEMLDKGKSYSGDEKINTS DNAKSCSGDEKVITSDNGKSYDYVKNESEEQEEKENMLNNKKRS **(SEQ ID NO: 30)**

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
p3	14	PFC0245c	**Predicted Protein Sequence** of 3933aa **(in underlined characters: fragment of the protein corresponding to peptide 14)** MIKKSEESKRLLRKKLNNDITNILLLFEKVQEWADLSNILQKLYLTIEKYELFVNVSSKF LLFRRLSQCLNPLLPSGVHSKALIIYSSIFKKVEMDFFINNIHILCSGIFEFMLHCTINL KTIYFKNIKSILRLKENVYIFAYALLLSLFNVVDSDNNILLYIYSINNYIGENIFFNNIW LLLLRHPEIRTNILNFLEASFSPQIYLLSKERIKMLLPYKDHLVLSSIIYCLNDKNILNQ RITLSLLINNFPLSHVPNKKNKKKIIDKSKSNDYHMDKPPYSPFNASTSSVILNNSNMDS MNDNRINENNNINNNDNKRHNIQINNDYLFGDMMNKQNDTTIMQSNKMLNRHNIIGEQHLD DDLLSSIHDDNSEKKNNNNFMLLNENNKISTSKEHLDNMHNRGIKSHEDIMGSNQNKMNL SNDEKDHWGGNLNSKVGNYDDKNICGKKHLGLKSEYGQVSYDESLERRLNNNNNNNNNNN NNNNNNGDNLKYQGSVDYDEHDISMSTENDKYGKMGNENMNDVFISKGKMMRKGYEDDGH HHININDDDNLNYDDNEDDEYGNYHNNNYNDRNYFNEYDEDDQYENNNNNNHSNNNNMLH LGSVDRNRRKQLKKKINNIGQTNNYDDDEEEEDEEEEDNNNNTSYNNNNNNSSSSSSIFF SDTSKKLIARNVIFLLKKSDIGLNRRIFKYLYLYESNDEKNFKDKEINFENYKIYCETII DILENKSDDNYNSIAEVIYILFKNKDYININRYIMEQVFLYLLNFCYKNREDTSIKSFLK NMLNLNLISYENILNIFLYTFYYLRRNDDLFVHYTNIYIKKYINLLNIMTFFVEFIKHMN KYLYIQFLFHFNLATLKLMNFLNLKIINIIKKYSHVKDLNEKYFIDSNDVVSGRHSTLYY FYFFVTHYNNYYLNKCLSIIVKDILPQNPSNRKMGNYQSHYYANNKHMLYMNTHEIHSAR MEEYSNKIQKVGFKNEIVDRKNKYDNNEYSESEIKMRAVDNSMNYIKRKVKKKNMESKDS SNSMSNMEINTNSTMANRLNHMQHIQHDGISSMEHMNNKINDNNNNNNVNYFFDGNNSNN NNNNNILENNNKLYFDKGYNGNYSKIENDQSFHNILMKYKFKLKQNLIDAIIKNHELYFF TNNCEYIIFLFYNYHLLIEKEKLNKSCFYFLKNILNNCTCENKNKFYFWCFLFLHIIRIN FNKSLLKNYKIKEAGDDTDDDDDDDDDDDDEEEDDDEDDDDEDDEEEDDEEDLGVDGLK NMSSKKGKKKKKKSVHKNKLMNKKYGRGGSSKYYYYLTSDKEIMLHGGMMGGVNYSDMEH DEDNLDVDDEDEQMFSYNKNKIRNKHFGELNKMKYMNEDITNNNNNINNNSNNNNNNKNN INNNNNNNNNNNNNNNNNLNNLNNFNNNVSINNGNNKYRNYFRSTEEELLFNKRFVEFLLP YSNNIAKYIFQYIKILKKNKKFIKLFFDINYLYFFCDNMFCLKILKKSLKCKDNHELTIN VKVILEYIINSKTNEYHIIHSNFYNLTHDVFKLYNRRNNLINYYLIKYIMRNKENLSYIF DNIIISMFDLITEIENLYEKIEMMKKDLSYSMMNNNDHVYDPNGMIEAADQRKSHYVSLK DNMNNMNNMNNVNNMNNVNNMNNVNNVNNVNNVNYHNNTINNNNNNNNNFSNHTSYVNEKT QENNYHNLMNTYEKYLKKLKCKFDYISYFFLNMENFMLWLYKHKISKKIYHSRNKMTLTN YEKNMAIIICYICTEGNINSFFFRNYLDVFFILFLKIIYLNENISELNNSANNIIQKEKN NLKHNSLLEFKRDTLSMLNNIFNINHNKKFEYMKILNLYYRQIIHHLLFLYYYFTVKKYY VLQLQLVHFIRYILPLYEKNIDKKFLTNESTEKDKVFKRMKYNNYEEFISASKYHFEIIN RNNYNMKNDVFIFSILRKSMTIIFNLNEQVLYKEVLKTIIDLIENIIDEKEVKNYYLTVF FLDLLYIIKMEDQKKKKNIFFIMKFCQFLIEIFKLIYRDEMKNELKCDKKFQDDNITIDI IENALSNNKMSTASFCTIFSIKTDDKFVNVQHKNISNLFSVIFNLYAFLKKKIKLYYKHN KHLINNDDKNNVVNNNSYIYYDNNSNVYNNNNTNIYNNNNNNENNAFNNNMPNNSNIMNN MNNMNDIYHISQHTNNNIRYNDVSSCGARGHNINSNENVNQNDLNNNSNYNYNRGMNNMN GDINNINGDINNMNGDINNMNGDINNMNGDINNMNGDINNMNGGNFKNPNSYNNNNNMNS YYSHNSSDYHKDNDNMRNNVNSSNSNFHNNNQDVQIMNRKNDSDGNVGNFDNNSTYSYMN NVNNDISMRLTNINNNNNININNNNNNNNNNLFAYNKNNPNALVNNMNNQDKPDQHNNSHQ YMYKENDMNQFGNSNYNNMDNTNNKFYNNYNYGKNVEGHTIYDNNNNNNNNSNNGAVNIN SVQGENNVMSRNNILFNHNVNNNEYYFSQKNEDNMASMNNNHHNNYHNNNNNHHNNNINN HHNNNINNHHNMNRNNNIYSEDSKTNECYSMREKMGDVNVAYNSNFYDDKNNYTHMKNDL IKNEQKNNYGFCNNHENTFIYNCKNRMNRNNYAFNMGSKKNKKIMLLKVCLSNIISINKL LYFITRPEFLFIDNLYSIFKDYIKNRNEYNKERLSKSDYYYEQREKLYKEHRRKMNRQNI RTDSSNNNNNNNINSNNNNNNNNNNNNNNNNNNNNNNNIYNNNNYYYSSSINKVSFDDDEKI EVESFLDHNGVVGSNKKIKREKIREYFKKEKNLLKKLNFMTKFSKNTIKKSMIVMNNSDE CIEKKKLSFSLTLLTEFDDVILIKIIDDLLNYYEKYKSKINLNEFMYFLFNIYLNICTLT KRIINHFIDFIFSFIKKITQTSQNIMSSLWFLYILFIIENNHIYVFNDKLQKKIIVEQIS ILIQISLYSYYSKNVKNNYNIQTPLPNFVQPFNIYYIIQNYFINNNYFHIKKNNKNIRYF YIKNLKLIEKNFNINDYSEIAAINALSFLLMCFYHTVNYNGTNKSYICESSVNIFYEHFS KYISLIYNNSLQNIFYRYVFLLIMNLLIDYNANSKYYIKKITFDLYSYITNVDIRCIKAL STLFKKLNETNIDELLLVPTSSIFSLKFNIINSRINYINKLSLIILAGNRNFYLCHLPKI AENISEYIKFCNDLKLYREILILICIIIIKNDENEIYIIIPTFISLILQIYHVERIKYKM AVENINNIDKDDDNYIYDFNSYNNKDVLSLLKTLLIIINILIKRNVSFINFYSWIFFKDI

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
			SIKKNRLEQQNREPGNLMIYPGHKTLVYNNKKKKQKNVVRYVSSSSDKDESSVYNISVDE ENSLKTQGRFFDDTYYKRKDNSGYTNKMKNFNSLTYEDKSSLMTGNQTSSTKDVGGMVNN AIRQNIEQNNMIHPNQINNNNNNNNNNNNVYNFNDFTNSMNQPNVINNNKKKKAFTTDDY FVKYDENQKVTKQTKLNHHNEDNLNDTITVYLNSNQEDYLYESKNNFTSIRSEHISSMVD IKKGSILNSNNILTNDNNTNNNIHSNIHNGSSSNNNNNNNSVCTGIKLDESKFVPFLDII ERIYSPNNILNKKYVSSEELKNEKSTRTYNSSLQEGSDYDEEEDEEYDVDADVDVDVDVD DDDDDDVDIVDVDVDDVVVDYNYYDNENNSVKIIDVDERKRSVHFYPQHLDGNTLKKNLY YNDNYLREYILSTKNELSGYSSFENNLSSSSVNSIKSNFSNTFSKDNINKNIITDDTSDD NDMMNSNNNMNSMMVPYNMHMTDDEFQENINNNNNNNNNNNNDNMYLSSDDGYPSQSNHKW IHFNSLLNYDIHELSKKKKKKKKKISIHSCKNLPLVLVYLSKKIKLNFYKYSMKKPKEET IILLKELNSVENDINDLFLEVDLNEVYYDFLIR **(SEQ ID NO: 31)** p14: GMNNMNGDINNINGDINNMNGDINNMNGDINNMNGDINNMN **(SEQ ID NO: 32)**
p13	66	PFL0250w	**Predicted Protein Sequence of 781aa (in underlined characters: fragment of the protein corresponding to peptide 66)** MSYNDGSEEENDSNTYIPDEKKKKKKKNNKNAYALINEVNYSQEDEQEVIHNTNSEDETS NRKGNGCVYSLSDQNVEDHIISLPEYLDLINNNNNNSNSYKMKKEKKKKKKKKKNQTDEE NMKNKKDHIYQNNITNQQNDIKNDYKKINHHNMNNKKNKIFCQDDQNIFNINHTFQIHET VQNNLIIPPSETCLASDIIQPSDTTQSNTYLNEATASQNDDNNNEDSSNEMGMFKKIFYR IKKIIVDKKGTPITNENDVDNDMCELNVMENNMNNIHSNNNNISTHMDDVIEDESNEEVF VINRNTDGYINTRENINVSTHVTRQMINLSELNPNDLLCNVSEYEEGQNINSLWNDNNNN NNNNNNNFLVGSLNALHPINHNLRNENIHNDNINNTHINYDNNNSYESPIHILSFSFKNI YNKISSYINEHITHIKEKIKKYWLERVQEANTQLNSPRPVHTRNTTNINTNININEDEND DPSCVQILFFMGLICKFPILWIIGSIVFCITPSEHRKTKTWSLVNTFFALLSIIYFITTT NFRLRKPTFFVILEQNVENKNTYPKGILKYNNMIHHKHIIIDQSSLHKWKDLHTNKVYKT SENYFLNRNFLSSQKPDSNILLSDTIYKLLNRIQVTVTFGKGNIYSSDNIEKIKPFFQNL RINMDPISYDKLTMTDEDIPDDFFGSGLRCERTYNNHNQNINEPQQNKEKEKWYLFWKEE EINNSHNKNIYNISIPVGEIFFFKSEYNCRIAFLYPKSILYDQNDIPSNFVEIQKIIIKP F **(SEQ ID NO: 33)** p66: MCELNVMENNMNNIHSNNNNISTHMDDVIE **(SEQ ID NO: 34)**
p8	9	PFB0145c	**Predicted Protein Sequence of 1979aa (in underlined characters: fragment of the protein corresponding to peptide 9)** MVFTFKNKKKKKEASSDKVSKESFNEEDNENNEKREKSDSWYKKIIETKGKSKTKYKNDN SLDDNINEDIINNNNNNNNDNNNDNNNDNNNDNNNDNNNDNNNDNNNENNNDNNNFNNYSDEIS KNIIHKDNELENQLKDTLKSISSLSNKIVNYESKIEELEKELKEVKDKNIDNNDYENKLK EKEDFVKQKIDMLNEKENLLQEKELDINKREKKINEKEKNIIKKEETFHNIEKEYLEKNK ERETISIEIIDIKKHLEKLKIEIKEKKEDLENLNKKLLSKENVLKELKGCVKEKNETINS LNDNIIEKEKKYKLLEYELEEKNKQIDLLNKQEKEKEKEKEREKEKEREKEKEKEYDTLI KELKDEKISILEKVHSIKVREMDIEKREHNFLHMEDQLKDLKNSFVKNNNQLKVYKCEIK NLKTELEKKEKELKDIENVSKEEINKLINQLNEKEKQILAFNKNHKEEIHGLKEELKESV KITKIETQELQEMVDIKQKELDQLQEKYNAQIESISIELSKKEKEYNQYKNTYIEEINNL NEKLEETNKEYTNLQNNYTNEINMLNNDIHMLNGNIKTMNTQISTLKNDVHLLNEQIDKL NNEKGTLNSKISELNVQIMDLKEEKDFLNNQIVDLSNQIDLLTRKMEEKENKMLEQENKY KQEMELLRGNIKSSENILNNDEEVCDLKRKLSLKESEMKMMKEEHDKKLAELKDDCDVRI REMNEKNEDKINMLKEEYEDKINTLKEQNEDKINTLKEQNEDKINTLKEEYEHKINTMKE EYEHKINTLNEQNEHKINTLNEQNEHKINTMKEEYEDKMNTLNEQNEDKMNSLKEEYENK INQINSNNEIKIKDVVNEYIEEVDKLKVTLDEKKKQFDKEINYAHIKAHEKEQILLTEME ELKCQRDNKYSDLYEKYIKLIKSICMIINIECCDDIENEDIIRRIEEYINNNKGLKKEVE EKEHKRHSSFNILKSKEKFFKNSIEDKSHELKKKHEKDLLSKDKEIEEKNKKIKELNNDI KKLQDEILVYKKQSNAQQVDHKKKSWILLKDKSKEKIKDKENQINVEKNEEKDLKKKDDE

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
			IRILNEELVKYKTILYNLKKDPLLQNQDLLSKIDINSLTINEGMCVDKIEEHILDYDEEI NKSRSNLFQLKNEICSLTTEVMELNNKKNELIEENNKLNLVDQGKKKLKKDVEKQKKEIE KLNKQLTKCNKQIDELNEEVEKLNNENIELITYSNDLNNKFDMKENNLMMKLDENEDNIK KMKSKIDDMEKEIKYREDEKKRNLNEINNLKKKNEDMCIKYNEMNIKYGDICVKYEEMSL TYKETSLKYEQIKVKYDEKCSQYDEIRFQYDEKCFQYDEINKKYGALLNINITNKMVDSK VDRNNNEIISVDNKVEGIANYLKQIFELNEEIIRLKGEINKISLLYSNELNEKNSYDINM KHIQEQLLFLEKTNKENEEKIINLTSQYSDAYKKKSDESKLCGAQFVDDVNIYGNISNNN IRTNEYKYEEMFDTNIEEKNGMHLSKYIHLLEENKFRCMKIIYENENIKSSNKIIGLYNY SRYYGLREDLCKEEIVPSKIGNISNKNENNNKKNNTCDGYDEKVTIVLCIILNEIIKFLF LNDEYVLLFEKIHKNVWKRMYIPEEIKFFILKYITLLNNLRDYIISVHNNMKNEKYDECW FLFQHYFERSSDVRKEMVHFLLERKSQENLISFKSKLKSKKEKILTMDILNFSKEHMQLK TIAHLRKEINYEKLSKDTLNRDYNLLLYKYQECVSKLKRVKNLMKEINQNVFIEKYDDIS KELDNFSDGYNEQNEQHVMDPILLNNNKNKNNKLITEHNNPIINRLTNFTQNRDSKYKNK IMDDVKQRKINSTMNNTNKNGINIIYNHYENLNKPNYNDNINRLNSYHQNIHIANSIHPN RNQNKSFLTNQANSTYSVMKNYINSDKPNLNGKKSVRNIFNEIVDENVNKTFVHKSVFF **(SEQ ID NO: 35)** p9: LLSKDKEIEEKNKKIKELNNDIKKL **(SEQ ID NO: 36)**
p15	45	PF11_0207	**Predicted Protein Sequence (in underlined characters: fragment of the protein corresponding to peptide 45)** MAKKKKQIHLNIIDFQKYYQTDDLLLDTSISTEKKTVDNQKFIRKNRTLEKDEVVQNIDW RTFDNEKEKETNNENTSNVNKIKSPGLEKKNFKKSNDVITLGARNKNKSTNLNADDIDFT NLRNKKKEDDIDFTNLRNKKKEDDLDFSNLRNKKKEEEDVDFSNLRNKKKEDDVDFSNVR NKKKEDDLDFSNVRNKKKEDDVNFSDVRNKKKEDDLDFSNVRNKKKEDDVNFSDVRNKKK EDDLDFSNVRNKKKEDDVNFSDVRNKKKEDALDFSNVRNKKKEDDLDFSNVRNKNKEDDM DFSNVRNKKKEDDLDFSNVRNKKKEDDLDFSNVRNKKKEDDLNFSNVRNKKKEDDLDFSN VRNKNKEDDMDFSNVRNKKKEDDMDFSNVRNKKKEDDLDFSNVRNKKKEDDLDFSNVRNK KKEDDLDFSNLRNKKKEESKENDTNKSEKPLYLRRLEEYRKKKKLESQANDTAMKMHEKE QIDDIQERKEEIKEEFKEEVKEEIKEIKEEIKEVKEEIKEEIKEEIKEVKEEIKEEIKEE IKEVKEEIKEVKEEIKEVKEEIKEVKEEIKEEIKEVKEEIKEEIKEEIKEVKEEIKEEVK EEIKEVKEEIKEVKEEIKEEVKEEIKEVKEEIKEVKEEIKEEIKEVKEEIKEEVKEEIKE EIKEIKEELKNDISSETTKEEKNTEHKKEETEKKKFIPKRVIMYQQELKEKEERNLKLLE QQRKEREMRLQLIRSKTQGTSSTFIPSAKLKHLESLKEEKKKEVKTNIQPKDNNNNNNNN NNNNNNIAVLKNNKNEEQNVIKKKSIFLEIAEKTENAKIVEKTDIEEIAKKKREELYKKQ LEKITKKNEEHLKYNNIYKHDVNIIKNFYNEIKDKIIQNYYFNQDDCISLCSILKTDDCN YMESHVPFYVVISIFMLSLPQKLQNDDYFKRASNIKNLLIYLKEVQFYPYKVKISTLIRR NIIN **(SEQ ID NO: 37)** P45 : EEIKEEIKEVKEEIKEVKEEIKEVKEEIKEVKEEIKE **(SEQ ID NO: 38)**
p25	90	PFD0520c	**Predicted Protein Sequence of 213aa (in underlined characters: fragment of the protein corresponding to peptide 90)** MRHKISENEIINKIDSINLKEVKDASACMNNYTNFISIKLKKNREGIIHSIQRIKHLEGL TKKLNKELSEGNKELEKLEKNIKELEETNNTLENDIKVEMNKGNLYKSRLALLKKNKVRI SKAQEIIDKDIIYMKSRINIMRENADKNNQKYDKIVSQKDKMHQEMEKFKKDRKNLQLNL KNTRKNHEFLKNKMQNLVLTMKKSTADDKRFQY **(SEQ ID NO: 39)** p90: TKKLNKELSEGNKELEKLEKNIKELEETNNTLENDIKV **(SEQ ID NO: 40)**

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
p2	12	PFB0145c	**Predicted Protein Sequence of 1979aa (in underlined characters: fragment of the protein corresponding to peptide 12)** MVFTFKNKKKKKEASSDKVSKESFNEEDNENNEKREKSDSWYKKIIETKGKSKTKYKNDN SLDDNINEDIINNNNNNNNDNNNDNNNDNNNDNNNDNNNDNNNENNNDNNNFNNYSDEIS KNIIHKDNELENQLKDTLKSISSLSNKIVNYESKIEELEKELKEVKDKNIDNNDYENKLK EKEDFVKQKIDMLNEKENLLQEKELDINKREKKINEKEKNIIKKEETFHNIEKEYLEKNK ERETISIEIIDIKKHLEKLKIEIKEKKEDLENLNKKLLSKENVLKELKGCVKEKNETINS LNDNIIEKEKKYKLLEYELEEKNKQIDLLNKQEKEKEKEKEREKEKEREKEKEKEYDTLI KELKDEKISILEKVHSIKVREMDIEKREHNFLHMEDQLKDLKNSFVKNNNQLKVYKCEIK NLKTELEKKEKELKDIENVSKEEINKLINQLNEKEKQILAFNKNHKEEIHGLKEELKESV KITKIETQELQEMVDIKQKELDQLQEKYNAQIESISIELSKKEKEYNQYKNTYIEEINNL NEKLEETNKEYTNLQNNYTNEINMLNNDIHMLNGNIKTMNTQISTLKNDVHLLNEQIDKL NNEKGTLNSKISELNVQIMDLKEEKDFLNNQIVDLSNQIDLLTRKMEEKENKMLEQENKY KQEMELLRGNIKSSENILNNDEEVCDLKRKLSLKESEMKMMKEEHDKKLAELKDDCDVRI REMNEKNEDKINMLKEEYEDKINTLKEQNEDKINTLKEQNEDKINTLKEEYEHKINTMKE EYEHKINTLNEQNEHKINTLNEQNEHKINTMKEEYEDKMNTLNEQNEDKMNSLKEEYENK INQINSNNEIKIKDVVNEYIEEVDKLKVTLDEKKKQFDKEINYAHIKAHEKEQILLTEME ELKCQRDNKYSDLYEKYIKLIKSICMIINIECCDDIENEDIIRRIEEYINNNKGLKKEVE EKEHKRHSSFNILKSKEKFFKNSIEDKSHELKKKHEKDLLSKDKEIEEKNKKIKELNNDI KKLQDEILVYKKQSNAQQVDHKKKSWILLKDKSKEKIKDKENQINVEKNEEKDLKKKDDE IRILNEELVKYKTILYNLKKDPLLQNQDLLSKIDINSLTINEGMCVDKIEEHILDYDEEI NKSRSNLFQLKNEICSLTTEVMELNNKKNELIEENNKLNLVDQGKKKLKKDVEKQKKEIE KLNKQLTKCNKQIDELNEEVEKLNNENIELITYSNDLNNKFDMKENNLMMKLDENEDNIK KMKSKIDDMEKEIKYREDEKKRNLNEINNLKKKNEDMCIKYNEMNIKYGDICVKYEEMSL TYKETSLKYEQIKVKYDEKCSQYDEIRFQYDEKCFQYDEINKKYGALLNINITNKMVDSK VDRNNNEIISVDNKVEGIANYLKQIFELNEEIIRLKGEINKISLLYSNELNEKNSYDINM KHIQEQLLFLEKTNKENEEKIINLTSQYSDAYKKKSDESKLCGAQFVDDVNIYGNISNNN IRTNEYKYEEMFDTNIEEKNGMHLSKYIHLLEENKFRCMKIIYENENIKSSNKIIGLYNY SRYYGLREDLCKEEIVPSKIGNISNKNENNNKKNNTCDGYDEKVTIVLCIILNEIIKFLF LNDEYVLLFEKIHKNVWKRMYIPEEIKFFILKYITLLNNLRDYIISVHNNMKNEKYDECW FLFQHYFERSSDVRKEMVHFLLERKSQENLISFKSKLKSKKEKILTMDILNFSKEHMQLK TIAHLRKEINYEKLSKDTLNRDYNLLLYKYQECVSKLKRVKNLMKEINQNVFIEKYDDIS KELDNFSDGYNEQNEQHVMDPILLNNNKNKNNKLITEHNNPIINRLTNFTQNRDSKYKNK IMDDVKQRKINSTMNNTNKNGINIIYNHYENLNKPNYNDNINRLNSYHQNIHIANSIHPN RNQNKSFLTNQANSTYSVMKNYINSDKPNLNGKKSVRNIFNEIVDENVNKTFVHKSVFF **(SEQ ID NO: 41)** p12: VDKIEEHILDYDEEINKSRSNLFQLKNEICSLTTEVMELNNKKNELIEENNKLNLVDQGK KKLKKDVEKQKKEIEKL **(SEQ ID NO: 42)**

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
p7	8	PFB0145c	**Predicted Protein Sequence of 1979aa (in underlined characters: fragment of the protein corresponding to peptide 8)** MVFTFKNKKKKKEASSDKVSKESFNEEDNENNEKREKSDSWYKKIIETKGKSKTKYKNDN SLDDNINEDIINNNNNNNNDNNNDNNNDNNNDNNNDNNNDNNNENNNDNNNFNNYSDEIS KNIIHKDNELENQLKDTLKSISSLSNKIVNYESKIEELEKELKEVKDKNIDNNDYENKLK EKEDFVKQKIDMLNEKENLLQEKELDINKREKKINEKEKNIIKKEETFHNIEKEYLEKNK ERETISIEIIDIKKHLEKLKIEIKEKKEDLENLNKKLLSKENVLKELKGCVKEKNETINS LNDNIIEKEKKYKLLEYELEEKNKQIDLLNKQEKEKEKEKEREKEKEREKEKEKEYDTLI KELKDEKISILEKVHSIKVREMDIEKREHNFLHMEDQLKDLKNSFVKNNNQLKVYKCEIK NLKTELEKKEKELKDIENVSKEEINKLINQLNEKEKQILAFNKNHKEEIHGLKEELKESV KITKIETQELQEMVDIKQKELDQLQEKYNAQIESISIELSKKEKEYNQYKNTYIEEINNL NEKLEETNKEYTNLQNNYTNEINMLNNDIHMLNGNIKTMNTQISTLKNDVHLLNEQIDKL NNEKGTLNSKISELNVQIMDLKEEKDFLNNQIVDLSNQIDLLTRKMEEKENKMLEQENKY KQEMELLRGNIKSSENILNNDEEVCDLKRKLSLKESEMKMMKEEHDKKLAELKDDCDVRI REMNEKNEDKINMLKEEYEDKINTLKEQNEDKINTLKEQNEDKINTLKEEYEHKINTMKE EYEHKINTLNEQNEHKINTLNEQNEHKINTMKEEYEDKMNTLNEQNEDKMNSLKEEYENK INQINSNNEIKIKDVVNEYIEEVDKLKVTLDEKKKQFDKEINYAHIKAHEKEQILLTEME ELKCQRDNKYSDLYEKYIKLIKSICMIINIECCDDIENEDIIRRIEEYINNNKGLKKEVE EKEHKRHSSFNILKSKEKFFKNSIEDKSHELKKKHEKDLLSKDKEIEEKNKKIKELNNDI KKLQDEILVYKKQSNAQQVDHKKKSWILLKDKSKEKIKDKENQINVEKNEEKDLKKKDDE IRILNEELVKYKTILYNLKKDPLLQNQDLLSKIDINSLTINEGMCVDKIEEHILDYDEEI NKSRSNLFQLKNEICSLTTEVMELNNKKNELIEENNKLNLVDQGKKKLKKDVEKQKKEIE KLNKQLTKCNKQIDELNEEVEKLNNENIELITYSNDLNNKFDMKENNLMMKLDENEDNIK KMKSKIDDMEKEIKYREDEKKRNLNEINNLKKKNEDMCIKYNEMNIKYGDICVKYEEMSL TYKETSLKYEQIKVKYDEKCSQYDEIRFQYDEKCFQYDEINKKYGALLNINITNKMVDSK VDRNNNEIISVDNKVEGIANYLKQIFELNEEIIRLKGEINKISLLYSNELNEKNSYDINM KHIQEQLLFLEKTNKENEEKIINLTSQYSDAYKKKSDESKLCGAQFVDDVNIYGNISNNN IRTNEYKYEEMFDTNIEEKNGMHLSKYIHLLEENKFRCMKIIYENENIKSSNKIIGLYNY SRYYGLREDLCKEEIVPSKIGNISNKNENNNKKNNTCDGYDEKVTIVLCIILNEIIKFLF LNDEYVLLFEKIHKNVWKRMYIPEEIKFFILKYITLLNNLRDYIISVHNNMKNEKYDECW FLFQHYFERSSDVRKEMVHFLLERKSQENLISFKSKLKSKKEKILTMDILNFSKEHMQLK TIAHLRKEINYEKLSKDTLNRDYNLLLYKYQECVSKLKRVKNLMKEINQNVFIEKYDDIS KELDNFSDGYNEQNEQHVMDPILLNNNKNKNNKLITEHNNPIINRLTNFTQNRDSKYKNK IMDDVKQRKINSTMNNTNKNGINIIYNHYENLNKPNYNDNINRLNSYHQNIHIANSIHPN RNQNKSFLTNQANSTYSVMKNYINSDKPNLNGKKSVRNIFNEIVDENVNKTFVHKSVFF **(SEQ ID NO: 43)** p8: IKTMNTQISTLKNDVHLLNEQIDKLNNEKGTLNSKISELNVQIMDL **(SEQ ID NO: 44)**
p9	76	MAL13P1.304	**Predicted Protein Sequence of 1792aa (in underlined characters: fragment of the protein corresponding to peptide 76)** MNNNNNNNNNSNNNNNSNNNNNGNSNNNFFSGKGNALSAYQNKILNIKSNNNNAHHFVNK NVPTYSPPNIIMANKKGGNFNNTSGNIINRYNVENNNHRNTYHPSNNNTRNSVNFLNKNI LYGNNNNNNNNNNNINITNISNNNNNINITNISNNNNNINITNISNNNKQPISSNQHPYQ QKQSHHHNNSINYNEYMDEKNMNTSQSIFKNMTIQRNSQQFNTSDFVNNINIMNAPHINE HSNIYKRNSLNIVNNAHIISNNMNIQSNRNSNISFPQNMNANIGGLKNSNHNLNNIEMKY NTLNNNMNSINKNTNITNVGTLNIQMKNNPMNVNINQNNYNTDFYVNENKVNSKNKENNN NHINIEKMNYIKSNVYLDNTLVQVNSNNNYNMDKNILNNNNNTYIINDKKNSTVNNNITN MDNNLVPGVMSSMNIPDDIKKRKKKERKKNENIYNNRNKSSINTEEHNNNIIDVANQNSE HFLQNNKQYGNITNIQNNNLSHDMNNYSINNSTTSDVIGIVELYKNSLSSKAVNKKKSKL IKDVIDDNKKRNKKEKKKTIPNNDSIINDMNKNKNVELLNETQIFDNKNYDKNNDIHNNI YNSNDNNLIHNKNNVNNDHTNIKEANNNNNRKSEHSEKNKDVHNYYYANNYQCITDEKNN KQYILWNNRTIEVTFVWLFITKEFNENRKKYTAFLPYLKHFYPNRLKDLIEQLEKYSLLK

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
			FNYIMHSSYNMQEEYNKNKEPNNINSNDNNNKNDDNNNNNNKNVDGNNNNNNNINSNDKE VLMNGMLLSDKSTLNSNKQIDNTLINNINSGFNNIIKNMSIDDNTIRSIMDNIENITKGK KKGRKKKQTLENNGDNIKEDIKSSKKDKKDNINDNNNDNNNDNNNDNNNDNNNDNNNDN NNDNNNNNNNNNNNNNNNNNNNNNHNNHNDNKNNQGDSKNEQEKKKKTRQYRKKSKITNDDN NEKIKQDNINSNNPKNDLKNNEIICSEEKNMKEDNIPDDTHYKEKRRNTFNLFNLDEGTI NMDLFNLSLLENDDALNKKENDMVSKSNIPSSFSSPPKETNNKNDIDKEQSDKHNNVQEF QNLNMNNEKSKDLYFNKNDIDNNDNKDKIINETSSGTFMQNLKETFYEKTKAMFSNLLSD TKISKDDELNNEVDQNCVKTSSGILNKEENNKKEDDEKHFDDNTNEQKKNVDNGEYNEMT AEPGRKKRKKDVLERKKKNLNKEIIKSEKRIRKYRTKKMLLKEAMEKGISNNIVESNITA NNNNDNNKNNDNDNNNNNNDNIINNNNNNGDMFSNSYDNSYIKENKYNKKLCFPQNNLLS DFRSEPIIIQQDKRKIIKINTINKIKRKYKKFRFCINKVFKKKSINDIIALNENIHKNKD LLTLFKKKDLANLKKKNLSFFMDTLKLEKIDMLIMKRIQMCLEKIKNTLLLTCTINNVQE IVNILKKAFEKRLYLMWPLIEFSNKYRLDQYFHLLGKNKNHINSSFKDTKLFVHQNISSL ILYFNQRSMDDKWVEYLKSQMKPKRRRRKTKMKEQFLEDKPIDYLNTMNSQHSNNFIGEN FSEIETVESKANEYAFVGYNQKRLLTQITPYDYRVVLNSNFCNKFFTPNWREQQSIFIDN LHFDMVPDTDEIKKHFENVYIRYMEYDEEKLRSKSDTKSKEHKKKDKKYKMLFKKKEGKG KPGRKKKIKLEIENVSNEIKIKKPRKKYERVKPRKSKNAMMNEEKSGNSEKQINNVLNVT NIENKHKSKKGRKPKESNLNNLNINEDINVAKASPDTLHRASLEFMNPNLFT **(SEQ ID NO: 45)** p76: GGLKNSNHNLNNIEMKYNTLNNNMNSINK **(SEQ ID NO: 46)**
p12	77	PF08_0048	**Predicted Protein Sequence of 2110aa (in underlined characters: fragment of the protein corresponding to peptide 77)** MNEIKSESLLQTRPFKLGIEDIQNLGSSYFIENNEKLKKYNNEISSLKKELDILNEKMGK CTTTTKIVEPAKTPEFTFWYYELKEMKGFQDLVMYEVKKKKKHFKVLSHSCLKYLSNREK MKIKKQEEEEKRLKLYSKNISSYMDVFWKKIEKLVWEEKKRELQQTLNKKKEMRFKKFVK EAIKKIKDARHNNAHELFENKYVSMSSNNNSEIVNNNASSVDNGDKELKEDDLTDQEEED YLLDEQMSSTDESENKEEEINMLDDEANLPIEELLKRMYGFKSGEDYINFMENEDDANEE NVIETSHNDEKSGDNSIGEDDNNNDEKGGDNNIDEDDNNNDEKSGDNSIGEDDNNNDHKS GDNNIDEDDNNNDHKSEDNSIGEDDNNNDEKGGDNNIDENDNNSDHKSEDNNIDENDNNS DHQSDQEQFNHETKDDIIKNSSYEHIDNKNYYNKTGEDYKSDKENYSPTRFHNKLKKEKY DEYDTKLKIEKREEENKNYEKDEHEYESDNYDKEKINKKKELILLKNDIENDSDETSEHI KRDSRSSCQKQNCEKKRRIIKDEYNLRRTKIAKSKPSSDNNNSENDNNNDNNNDNNNDNN DDNNDDNNDDNNDDNNDDNNDDNNDDNNNEHKNDSDDNDDILTCNMDEKHLTKIPPIIKA TLRDYQHAGLHWLLYLYKNNINGILADEMGLGKTLQCISLLSYLAYYFNIWGPHLVIVPT SILINWEIELKRFCPCFKILSYYGNQNERYKKRVGWFNKDSFHICISSYSTVVKDHLVFK RKRWKYIILDEAHNIKNFNTKRWNIILSLKRDNCLLITGTPLQNSLEELWSLLHFLMPNI FTSHLDFKEWFSDPLNLAIEKSKIHHSKELIDRLHTVIRPYILRRLKKNVEKEMPNKYEH IIKCKLTRRQQILYDEFINNKNVQNTLNTGNYIGLMNILIQLRKVCNHCDLFTNKYIQTP YYYMLSIRYFVPRFFILFEKNYYADFYLILFLHNEFTSLGGRDVTKETSPSSKSFDLAHI LTKHNTNELYDNNHISELYDNNHISELYDNNHISELYDNNHISELYDNPMSHKNYKHNSN GYTYPNDPINNMNNNPSGFTKTSEQFGQIVSHERDNNYHMMDHNNMNNLLSKEMVNSLRN DDNSNNNFYKYSLTSNNNDSQTSIHDNKQCDYNKLCADTFNNINSIGNEEKRSLNVLNEQ NNNNSKDNNNNIDNNNNIDNNNNIDNNNNIDNNNNIDNNNNIDNNNNIDNNNIDNHHNNNQHCN YNDNWPSDYPTNIINHRNAFLSILKLLNQSNPLNNDNNNNNNNNNGNNNIYNMNRYNSRN SRNSSLSNIFSSNTSKMNSFQLDFLYTNSFINQDALCKNSFFVNINIEDVHSYIYNSIYK EYIPKNILSFSDEFLTELNNNYDILSLYIDPYNRYKSYNEYLYKMKEEGTLTNQQSLGDI NNKHIYHKSTSNENTHMKNRKTFIYKYNNMFKVINNDTQYQNIFTDDTNNSYYNSLEHNL WIKRNQIDERKKEEEEEQNKYYNVCMNNLYILRNERIPIFGKNFLDLIKKEFTKDKNIVY NYTNNVPIDYYSSVKEVWVEDICEKDNKKRKCKREKRWYKKIKKTNNPPEDSEVYRENSS DVEKYNCDVEKDNCDDEEKDNCDDEDMNSNLSSNVYGCIDISSQNFIHSRYHNPMMNMSY IIEFLFPNMEQFLKRHEKMIHNFTLINNPSVICKSHDIRINNNLLNYSNDKMNPIILQIK NATRVYHDAFLKQSIIFPLNKDISLGSGKLCALEKLLSKCKREGNKCLLFTQFIKMLDIL EIFLNHLNYSFIRLDGSTKVEQRQKIVTKFNNDKSIFIFISSTRSGSIGINLTAANVVIF YDTDWNPSIDKQAMDRCHRIGQTKDVHVFRFVCEYTVEENIWKKQLQKRKLDNICINMGN FNNSNTHSKITDTDPTHNKDWFTNVDTIKEVFINKKNNDDDDDMYKDRLLHEQVENKDKM

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
			NVRFEKTLEHVEDKDDIRALNETKKETQNEISQNMQVSTNKKNIYIYINILYLNVNFFNK HPLGEFTTRNDFQDSYNLTSYCFNFLNENLTDSLKQQIDEMRMKIEIEMMNTGDENMSLS DLSNKSHNSE **(SEQ ID NO: 47)** p77: EKLKKYNNEISSLKKELDILNEKMGKCT **(SEQ ID NO: 48)**
p20	79	PFB0315w	**Predicted Protein Sequence of 1712aa (in underlined characters: fragment of the protein corresponding to peptide 79)** MDSDKYKKFYVYNHGFTKQPFYERNLNDKGIHLKELKRLERVDEPRLYNNVDKIPNKKEI IYNNIKSNNIQVRVNQNNNEEKKKEEANYTCVNNKYVTLKNKVHVNKYVNNSNINKIKIV PIIKCSNYKIKNNPISHLKSNYENKFVKLSNFSNIKNGCSHKDNVINETMDQHKSEQLNN DNIKKLLYDYCIFREDTIKTKTNISYNKMNSFKDNEENINYMDNNNIKSNSSSYCSYSNK INQNNVNHTHLKTEFLNEKNSHTQNEQSIPLLDGLQNNHNSATKFHNNIYDNNNSLVNYK SDKGIDLHNKMMKIETDKNGIITLEKKKHDEKYYNNIFLNPLNDNSNNVVITTCDNKESY RNSTSDMINKIFEKMMNEKKNILKMKNFNDVIKKKITMAKEKILNSNSTINMKKVSFYNS KDEDLFNEKENSYKYGVKRENQEDINVIKNNMKRNNININDNNDNINIIKNDSVSKNIHIN NKKKRDDDFPFNNSAGLLLDFDLCKRKVLEILKNVQSSKKKNKILTNHNHSSDNQNCHSS DNQNCHSSDNQNCHSSDNQNCHSSDNQNCDSNACNKKDEEKKRKKKKIKKKNKMKNKSNN KSKNKRETKSKKISNNNNNDNMNNQCDNMGDQRINNENMDKQNVNIQNEGNGFNNNKNNN DLLNVYISPNMINHSLSSTCEKKNKEDNKMNDNKFLNSSSKMKIPEISTNNSNEKIVNVS NDEMLVYHNLTVLNVKEQGGVTEESSCIKRTYFVDQFYDSYNMRNEKITDDNMQVEDIYN VKENIKRTLKGDGHDDVKTNMLSEDNSYASGLWGNEINFISNNENCLNSYDISCDEKYIP NEEEQDEELCSNNILVKDIEEKKMCGKLFFEEICVFRINEKNEHGHENLRKNNHNDDTHK MYSSYENIQNINKQSTNPFCKKDEMEKSQGTNLFYDNYINSVDITKLELNKNCYQHINYE VQNLIKKENSYAAEMNVGLVFRKYIPILINLSCNYLLIKKNEKNVITCISYTNIIDVKIV KKSKKNKERFLFKIVYVFKKKEQKTEKNVTLLFRANLMEIFEKIKGRVDYCIIPNEDDKN IQLQDKKKKKGKKKKELQEEKMKKKKKTQEYVDIETVYEYVIEKYKRVHVLYLGRLLQIV EKLFKKYILKYSFHKLRIFYEYKIEMEKLKKNYIHCIYDISDKLEFLIKKKMQHYFNHII INSYESSFINYQIKTNDMLYNLLLKEKSAYQNHLGKNYILILYKVLLSMYKKKMAIYFRS FVYNNIKVSKKKNAFAYTLTRVNSILVLYERRIKSFIFSKLKFNYDNVSYFCFTMYKIYL RRILFGYLRIRDNRINIKNVIEKNVYRLVKLISKISDNHKYNAFLKLQKYVYEQNEKKNK MICDNLIYANNELCNNLDKIAIEKGINQIDCLIKFKRKECLMKYFYTLKGPQINTERFYY CIRYCSIFSFVLNKIIQKKVQHIFFQFVLKTLQRNNKNRLTHAIKLLQVLVQKKEKKSVI DVLQLYDKYPYIFQYKDLTKIEVFVICVQNFVTLYNRKLLLNFLLKLHYLKYQEQFMKTY NGIGSIYKFVHVLDKKLMNTIRESFRVILQNDKFLREKMNMKMEQMDMKMEKIDVNMDQM DVKMEQMDVKMEQMDVKMKRMNKKKSKQIHVNYNNKAYSSSSPSPMLRYNKYKDMSSNSA SLIKKYPFLIYNSEISPDCTTMAGKFYNQKNK **(SEQ ID NO: 49)** p79: EKMNMKMEQMDMKMEKIDVNMDQMDVKMEQMDVKMEQMDVKMKRMNK **(SEQ ID NO: 50)**

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
p21	80	MAL8P1.12	**Predicted Protein Sequence of 1033aa (in underlined characters: fragment of the protein corresponding to peptide 80)** MSEESFDDTNKAFENEKDIILEKIVKDENNLNNCSNMINMDDVENMKKELYVLHKKDEEI ENNVDCFSGDKYNVENVINLKKKKKKDEDTDSSYYKTTLDEVYDTSDISTDEMLSNYSSS EDNNNIEMNIINDFYLKDDNTYCLEWNSDIINVLSEEIKEKEKLLEDENKDICNMKSRFL KLEKYVNIKKKKIINIKKNIEEKRKIEFDEKEIFKCLQIKNDFLKKENKKIELEREKNNK KIIETQNNITTCQKNIDDIKKELILKENELNDFINKIKIIQQEEYEIEKIKLSKDKEIQN VSYNLEKYNNEKIQQDKKYEQVKMNNMKFDIELKSIIQEYYDIKKDIKNISNKYICIMDM IKCRDKTIYKFEKDYTKTIHKEKQLQNKCLHKQNLINTQKDKNIILNNQIKKIQFDINKI RKELNDKQMSYDKTIIDRDHLNKEYEYEIVEIKEKLQEEKKSLENTLQHLNETYITMSTN YEESKNEYEKEQVNNIEKNDLIKSSEQILVQLQNKLQKLLDEIKSLDLEKFQLTQTLQVI KNDYITLEADVLGTQIKIKQIKSNIKKTEKELERQKEMLYKFDFQTQVLTKKINMISGIS TFEKKKENQKKIILLEKELYKNEDIYNTLNNEMKRINIEIKNIKLYQNELQEQKMNYKNL YEKLQLEIKSLESTINNEIKEKENIMLIELNLKIELDKLKSTFSKHVDNLNICKKEKKEN MNNAKLSEQDINAHMESLKVIIKNINDEIHKLNIQLYEKKNKSNNLQLKLNSIIICNQKN KDQKDICPNENQHIYYKMKIDQDIINLKEQLKKINEQIDKENIETKNFQRTLDDIIQTNK EFNDNIKSIDPQYKILLKK<u>KNKLNKKWEQINDHINNLETNINDYNKKIKEGDSQLNNIQL QCENIEQKINKIKE</u>SNLKVENNINDLFIKIERASNQLKKNLAPTTNMMKLKNKQIKDDEN NLSNNNNNNNNNNNNINVNVNVNCEPVPLEKHIFKQIQMESLKEKLSLLMECFKNNIDNV IMKEVFNLIETAE **(SEQ ID NO: 51)** p80: KNKLNKKWEQINDHINNLETNINDYNKKIKEGDSQLNNIQLQCENIEQKINKIKE **(SEQ ID NO: 52)**
p23	83	PFC0345w	Predicted Protein Sequence of 1711aa **(in underlined characters: fragment of the protein corresponding to peptide 83)** MNNITIRKPLFEVPNENKSNVLKYEKDNDFNNKKNDPSNLESYISSTLPYKRIENNHHNY NNAKYDENNKNDDDHIPLDLNNKENMNFFVNKKNIHNSNLNYNHDNILQSYRNGEINRNY NIMDNMYDVYYINKSKANLNDYLKHVNINHTAPCIGEFRTCMNCFLNISTLFCKTCNIFL CAICNVKLHNNKSNHIINVASSGLYENNVKFNDIILKEKDKWLVELDNSIPIKIREKCSV HTKEYIKYVCKTCKYTLLCADCLLNDPVHV<u>QNKMENDMNIIKNDMNIMENDMNIMENDMN IIKNDMNIMEKDMNIIKNDMNIIKNNMNIIKNEMNIIKNV</u>PEQKRKNEHFLPEQVQENND NKNGSKNDKNLKDSNKKKRENQYIVSIYKKEETSDSNNKDIIKDVIYNNDIDKLKPGFKL IRGNHEILTLIDARNDIKEELNNKLEILCKKSLILKNTLPSLRNICKYGKITCKNNKRSI RSGFTVTNNILNDKKVKIHNDLKKLQDKSTNFLKKLDQERINYRNYLEKKKSELQHMIKL SNKNAGLALDYYVQKLESFKCLFFTKDNLIDIEKKLEVPHSKIKSEFLSFLIEEMKYDIL NSKMNIQNRCQSITKEFEQLFNCNIEIPVYPVHFRDFLKKRTFNNKQDVHLISNDKKKKQ QYFHILPFTDFYMNIEISYQIKCKRKDSLHSKWEKRTVSVRSIYLCIHTHSRYIKRSNKY QNDEFDENVSHKNDAVGSIAYEMEQNEINEQERRDGEMLGVDEMENRNKIENYEHIDNAS SEISNKENCLIQKNMSNNLSNDIESIICLSNVEIKMFNDPNITNITILEKRNYSYGIELT EYNDKKDLVGYWLLSQNNEKDMKELYHILCAIKKKNPKAARIPSFYPKINMNNSMFNYHE NNISTIYKNFSANLIEPSYFINTSEHEKDERDGKYLEASINDYMSDDKKKKRYDSIESLR GSDKIKNDQIYQGGHSSSLLYYYDNNNDDNNNNMYDSSSSSNHNYYILTNDKRLNMDNFI NNNLEINNSQNKVIEKNLEYINNVKLTKTSNYEQSNNTNSKDEHNISSDKSKKEDTLNLS RKSSYEYNNKILQSTSNKSLNGAYENNLFSGKKKNKGTVLKDIEHINDIQDKYPEDLNI NCVNKYVIENEEKHLLPLELEYNLVSSDEKFGLNKIKNDNNIIYMKHQNYHNLYDDNQKK HILFDTNKNVSIQRNNNINSVIKTNHYEVEKNNKDQRNYDNFTCDKKKKIYYNIINSDKD IYHNNIIYTKNEKEGIGNIHLNRNDKDITNFELLKLDGVKEFLDTFKDSYIDCHNKKENI LNMTNKNKEDHQIIDVADKIFNETNMITMDNNKIYDDKNVHEKKCTHNDVIHHNMDILST SIKNNEENLFIDTYQKQNRIGDIYMNRINILQEDDDDDNHNNNHNNNNNNNKLILFEYTK NDQMLHNNKNNLEGTEEFSDFIEKKNKIKIKNKNESYHKIDESLLSNEKNNKVSLLLINN NKDSSSVDNNKNNNNKNNNNKNNNNENNNKNNKNNNNDSFSKDNNLINNDNNNNNNNDS FSKDNNLINNDNNNNNNNNNKVIKKEIIDDKEKNDIHKRDNIYIKDVSVSPLINNHPNLN SMRKDRTIEPLKIINGKNKLIKDLKKIQEQVERKIRKYKIQMDQENKKPPPSKNKINMKS

N° Parmed	N° GPC	Name of the gene in PlasmoDB	
			INLDIDDDQNVDSQGIVDYVLNQIGNKKMGQ **(SEQ ID NO: 53)** p83: QNKMENDMNIIKNDMNIMENDMNIMENDMNIIKNDMNIMEKDMNIIKNDMNIIKNNMNII KNEMNIIKNV **(SEQ ID NO: 54)**

[0221] The latter was performed using all the data in a multivariate analysis (following the methodology described in Roussilhon et al. 2007, PLoS medicine 4(11) e320 1791-1803). Please see Table 3 below.

[0222] In the multivariate analysis, when age was systematically controlled for each statistical test (Table 3 below), antibodies to peptide **P27** were high when the number of malaria attacks was low after 1 and 3 years of active and daily clinical follow-up of the villagers. Indeed after 1 year, high anti-**P27** IgG1 antibodies were associated with a low number of malaria attacks during the first year following the sampling (F ratio= 8.92 and p= 0.0047). After 3 years, high anti-**P27** IgG1 levels remained associated with less malaria attacks observed during the 3 year period following the sampling (F ratio= 12.14; p= 0.0012). Similarly, after 3 years, absence of malaria attacks was found associated with high anti-**P27** IgG1 response levels (OR= 13.17 [1.27 - 195.84]; p= 0.0408.

[0223] In the same conditions of statistical analysis applied for other peptides, anti-**P27A** IgG3 were found at high levels in individuals presenting with a low number of clinical attacks (F ratio= 8,33 and p= 0,0062 after 1 year of survey).

[0224] When anti-**P27A** responses were tested among a higher number of Ndiop inhabitants (n=102), the association between high antibody responses and reduced malaria attacks was confirmed. After 1 year: F ratio = 13,27; p=0.0004 when using the number of malaria attacks as a continuous variable; OR= 42,55 [4,05 - 610,07]; p= 0,003, when malaria attacks were tested as a dichotomous variable (presence or absence of attacks); and after 3 years: F ratio = 27,84; p<0.0001 when using the number of malaria attacks as a continuous variable, Odds ratio (OR)= 68,69 [4,27 - 1717,64]; p= 0,0051, when malaria attacks were tested as a dichotomous variable (presence or absence of attacks).

In non linear multivariate analysis (when testing the occurrence of malaria attacks as a Poisson distribution), anti-**P27A** IgG3 (p= 0.002) was present at high levels when the number of malaria attacks was reduced.

Table 3:

[0225]

F ratios were calculated with regard to the number of malaria attacks identified 1 or 3 years after the blood samplings:

Anti-peptide IgG:	F ratios (1 year):	*p* values (1 year):	F ratios (3 years):	*p* values (3 years):
IgG3-pMR198	5.43	*0.025*	1.29	*0.262*
IgG1-p12	5.57	*0.023*	2.11	*0.154*
IgG3-p14	6.77	*0.013*	2.5	*0.122*
IgG3-p8 (LR148A)	7.57	*0.009*	8.14	*0.007*
IgG3-p9	0.645	*0.427*	3.74	*0.06*
IgG3-p76	2.71	*0.107*	4.25	*0.046*
IgG3-p77	7.17	*0.011*	3.31	*0.076*
IgG3-p66	11.62	*0.0015*	3.96	*0.053*
IgG1-p27	**8.94**	***0.005***	**11.74**	***0.001***

(continued)

Anti-peptide IgG:	F ratios (1 year):	*p* values (1 year):	F ratios (3 years):	*p* values (3 years):
Igg1-p45	2.26	*0.14*	1.47	*0.233*
IgG3-p79	2.9	*0.096*	4.45	*0.041*
IgG3-p80	3.25	*0.079*	4.19	*0.047*
IgG1-p83	9.06	*0.003*	14.54	*0.0002*
IgG1-p90	4.41	*0.042*	0.88	*0.354*
IgG1-pAS202.13	3.22	*0.0803*	9.22	*0.0042*
IgG1-pLR179A	3.4	*0.072*	6.88	*0.013*
IgG3-p27A	**13.35**	***0.0004***	**28.55**	***<0.0001***
Due to the number of tests carried out, individual p values ≤ 0.0014 still remain significant in this Table. Numbering corresponding here to Giampetro Corradin indications.				

[0226] Table 3: The antibody responses found in Ndiop were tested in multivariate analysis by stepwise regression. The number of malaria attacks identified during the 3 years following the serum sampling were used as a continuous variable. Both age and Log10-transformed antibody responses were simultaneously tested as explanatory variables. The F ratio, (i.e., the ratio of the mean square for the effect divided by the mean square of the error) was calculated, and F statistics were used in order to determine that the effect test was null. By testing the hypothesis that the lack of fit was zero, the F test indicated if all parameters of an individual effect were null. The probabilities indicated in the Table correspond to the significance levels determined for the F ratio values (i.e., the probability that given that the null hypothesis is true, an even larger F statistic would occur due to random error). Using Bonferroni correction (i.e., taking into account that 18 different statistical tests were carried out), individual p values ≤ 0.0028 can still be considered as significant.

- **Antigenicity/Immunogenicity**

[0227] The peripheral blood mononuclear cells (PBMCs) of 9 out of 17 (53%)* adult Nigerian donors proliferated when stimulated with **P27A,** with a mean stimulation index (SI) of 4.0. Two of the 17 (13%)* donors also gave significant T cell stimulation with **P27** (mean SI = 3.1).
*SI is considered significant when > than twice the negative control

- Antigen size and solubility

[0228] **P27** and **P27A** are 27 and 104 amino acids long respectively. They are readily soluble in aqueous solutions.

- **Antigenicity:**

[0229] Peptides **P27** and **P27A** are immunogenic, both alone and in combination, in 3 strains of mice (CB6F1, C3H and ICR) when given subcutaneously with Montanide® ISA 720. Table 4 shows ELISA titres and numbers of responding mice/number immunised with **P27, P27A** individually and in combination. Each mouse was injected with 20 μg of each peptide together with Montanide subcutaneously at 0, 3 and 8 weeks. Antibody titers were assessed 10 days after the second and third immunizations. No significant increase in antibody titers was observed between the second and third immunizations.

[0230] Strains of mice, such as CB6F1, C3H and ICR, are commercially available, e.g., from Charles Rivers Laboratories, Harlan, Taconic. For example, ICR mice are available from Taconic (Corporate Office USA Taconic One Hudson City Centre Hudson NY 12534) under reference IcrTac:ICR.
C3H mice (haplotype $H2^k$) are available under strain code 025 from Charles River Laboratories, and CB6F1 mice are available under strain code 176 also from Charles River Laboratories (Charles River Laboratories, Inc. 251 Ballardvale Street Wilmington, MA 01887-1000).

Table 4:

	Peptide 27		Peptide 27A		COMBINED	
	Mean Ab titer ± SD ($X10^3$)	No of responders	Mean Ab titer SD ($X10^3$)	No of responders	Mean Ab titer	No of responders
CB6F1	16.7 ± 10.5	4/4	24.3 ± 0	4/4	ongoing	ongoing
C3H	145.8 ± 118.8	4/5	182.2 ± 72.9	4/4	218.7 - 182.2	4/4
ICR	10.8 ± 9.4	3/5	ongoing	ongoing	ongoing	ongoing

[0231] Weak responses were obtained when mice were immunised with **P27** and alum or GLA; however, alum is known to be a weaker adjuvant in mice than in humans. Assays of the immunogenicity of **P27A** with these adjuvants are currently ongoing.

[0232] The sera of mice immunized with peptides **P27** and **P27A** in Montanide® ISA 720 localize the malaria antigen to the cytoplasm and the periphery of infected erythrocytes in IFAT.

[0233] In Western blots of lysates of infected erythrocytes, these antibodies also reacted specifically with a protein of the expected molecular weight (about 130kD), as well as with a small number of bands of lower molecular weight, which may represent degradation products

- **main conclusions:**

[0234]

- The putative random coil segment of ***Pf*27** was selected and chemically synthesized as **P27A. P27A** was recognized in ELISA by 76% of adult sera from Burkina Faso, Tanzania and 86% in Ndiop, Senegal;
- Human purified antibodies specific for **P27A** also stained the cytoplasm and periphery of infected erythrocytes. Their activity in the ADCI assay was high, i.e., comparable to that of **P27.**
- T cells from 9/17 adults living in endemic areas responded to **P27A** (average SI =4).
- Longitudinal follow-up study in a malaria-endemic region revealed that high titers of antibodies against **P27A** were associated with clinical protection from malaria.
- Peptides **P27** and **P27A** induced high-titer antibodies in CB6F1, C3H and outbred ICR mice when injected subcutaneously, alone and in combination, together with Montanide ISA 720 adjuvant. The sera of these mice also reacted specifically with the cytoplasm and the periphery of *Pf*-infected erythrocytes in IFAT.

Claims

1. A polypeptide, the amino acid sequence of which is:

- the sequence of SEQ ID NO: 6;
- the sequence of a fragment of said sequence of SEQ ID NO: 6, said fragment having retained the sequence extending from position 223 to position 326 of said sequence of SEQ ID NO: 6; or
- a conservative variant, which derives from said sequence of SEQ ID NO: 6 or from said fragment of SEQ ID NO: 6 by at least one conservative amino acid substitution and/or at least one conservative internal amino acid deletion, provided that said conservative variant has one of the following properties:

a) said conservative variant has retained an unstructured or unfolded 3D-arrangement, said unstructured or unfolded 3D-arrangement being as defined below, e.g., as assessed by the Hot-Loops predictor of the DisEMBL-1.4 software, the six parameters of the software being left at their default settings;
b) said conservative variant has not acquired a globular functional domain (such as a zinc-finger, knottin, animal toxin, FGF molecule, chemokine), nor a structural motif of protein made of tandem repeats, such as an alpha-helical coiled coil domain;
c) said conservative variant has retained the property of inducing IgG1 and/or IgG3 antibodies, more particularly, specific IgG1 and/or IgG3;
d) said conservative variant has retained the property of inducing antibodies that are specific of the Plas-

modium-infected erythrocytes, but not sporozoites;
e) in the ADCI assay, said conservative variant has retained an inhibitory effect on *Plasmodium* growth that is of at least 92%, e.g., it has retained the property of inducing specific IgG1 and/or IgG3, wherein said induced IgG1 and/or IgG3 have a SGI value of at least 90%, preferably of at least 92%, in the ADCI assay;
f) said conservative variant has retained the property that, in humans under natural exposure to a malaria parasite, the total proportion of individuals having IgG1 and IgG3 antibodies that are specific of this conservative variant is higher than the total proportion of individuals having IgG1 and IgG3 antibodies that are specific of prior art peptides or polypeptides, including peptide P27 (SEQ ID NO: 14); more particularly, the proportion of individuals having IgG1 antibodies that are specific of this conservative variant is higher than the proportion of individuals having IgG1 antibodies that are specific of prior art peptides or polypeptides, including peptide P27 (SEQ ID NO: 14);
g) said conservative variant has retained the property that, in human beings under natural exposure to the parasite, it induces specific antibodies (IgG1 and IgG3) that are very strongly associated with a state of resistance to malaria;
h) said conservative variant has retained the property that parasite-induced antibodies, which are specific of said conservative variant, are present in individuals, who resist to malaria and are absent, or are present at lower titers, in individuals, who have malaria attack;
i) said conservative variant has retained the sequence of SEQ ID NO: 10, or comprises an ortholog variant sequence of said sequence of SEQ ID NO: 10 thereof, said ortholog variant sequence having at least 98%, preferably at least 99% identity with said sequence of SEQ ID NO: 10 over the entire length of SEQ ID NO: 10, said ortholog variant sequence consisting of 102 to 106 amino acids, preferably of 103 to 105 amino acids, advantageously of 104 amino acids.

2. The polypeptide of claim 1, wherein said conservative variant has said property listed under i) in claim 1.

3. The polypeptide of claim 1 or 2, wherein said conservative variant comprises the sequence of SEQ ID NO: 10, or comprises the E292G ortholog variant of said sequence of SEQ ID NO: 10, i.e., the sequence of SEQ ID NO: 12.

4. The polypeptide of any one of claims 1-3, wherein said conservative variant is a variant by at least one conservative amino acid substitution.

5. The polypeptide of claim 4, wherein said at least one conservative amino acid substitution is the E292G substitution.

6. The polypeptide of any one of claims 1-5, wherein the sequence of said conservative variant is the fragment of an ortholog sequence, said ortholog sequence being the ortholog of the sequence of SEQ ID NO: 6 in a *Plasmodium falciparum* strain other than the 3D7 strain, said ortholog sequence being encoded by chromosome 6 of said other *Plasmodium falciparum* strain.

7. The polypeptide of any one of claims 1-6, wherein the sequence of said conservative variant is the fragment of an ortholog sequence, said ortholog sequence being the ortholog of the sequence of SEQ ID NO: 6 in a *Plasmodium* strain other than a *Plasmodium falciparum* strain, said other *Plasmodium* strain being preferably selected among the strains of *P. vivax, P. ovale, P. malariae, P. berghei, P. knowlesi, P. chabaudi, P. yoelii,* said ortholog sequence being encoded by chromosome 6 of said other *Plasmodium* strain.

8. The polypeptide of any one of claims 1-7, wherein the sequence of said fragment or conservative variant consists of less than 844 amino acids, preferably of 70 to 150 amino acids, more preferably of 80 to 150 amino acids, even more preferably of 85 to 140 amino acids, still even more preferably of 85 to 120 amino acids, most preferably of 90 to 115 amino acids, for example of 104 amino acids.

9. The polypeptide of any one of claims 1-8, wherein said conservative variant derives from said sequence of SEQ ID NO: 6, or from said fragment of SEQ ID NO: 6, by one or several amino acid substitutions, wherein said one or several substitutions do not result in increasing the sequence identity score, said sequence of SEQ ID NO: 6, or said fragment of SEQ ID NO: 6, has with respect to human proteins, respectively.

10. The polypeptide of any one of claims 1-9, wherein said conservative variant of the invention does not comprise a higher number of Asparagine and Glutamic Acid than said sequence of SEQ ID NO: 6, or than said fragment of SEQ ID NO: 6, from which said conservative variant derives.

11. The polypeptide of any one of claims 1-10, wherein said conservative variant does not comprise a higher number of highly hydrophobic residues, preferably not a higher number of Isoleucine (I) and Valine (V), than said sequence of SEQ ID NO: 6, or than said fragment of SEQ ID NO: 6, from which said conservative variant derives.

12. The polypeptide of any one of claims 1-11, wherein the sequence of said conservative variant of the invention has at least 70% identity with said sequence of SEQ ID NO: 6, or with said fragment of SEQ ID NO: 6, from which it derives. Said identity score is computed over the entire length of sequence of SEQ ID NO: 6, or of said fragment of SEQ ID NO: 6, respectively.

13. The polypeptide of any one of claims 1-12, wherein the sequence of said conservative variant is the sequence of SEQ ID NO: 18 or NO: 22 or NO: 26, or a sub-fragment thereof, which has retained the sequence of SEQ ID NO: 12.

14. The polypeptide of any one of claims 1-13, wherein the sequence of said conservative variant consists of the sequence of SEQ ID NO: 12.

15. The polypeptide of any one of claims 1-14, wherein the sequence of said fragment is the sequence of SEQ ID NO: 16 or NO: 20 or NO: 24, or a sub-fragment thereof, which has retained the sequence of SEQ ID NO: 10.

16. The polypeptide of any one of claims 1-15, wherein the sequence of said fragment consists of the sequence of SEQ ID NO: 10.

17. The polypeptide of any one of claims 1-16, for use as an active principle in a medicament.

18. The polypeptide of any one of claims 1-16, for use as an antigen.

19. The polypeptide of any one of claims 1-16, for use in the palliative and/or curative treatment of malaria.

20. The polypeptide of any one of claims 1-16, for use in the palliative and/or curative treatment of malaria in an individual selected from infants, toddlers, children under the age of 5, pregnant women.

21. An antibody, which specifically binds to the polypeptide of any one of claims 1-16, or a Fab, a F(ab')2, a Fv, a Fab/c fragment thereof, or a scFv thereof.

22. The antibody of claim 21, which is an IgG1 or an IgG3.

23. The antibody of claim 21 or 22, which is a monoclonal antibody.

24. A hybridoma, secreting the monoclonal antibody of claim 23.

25. The antibody of any one of claims 21-23, for use in passive immunotherapy of malaria.

26. An immunogenic or vaccine composition comprising at least one polypeptide according to any one claims 1-16, or at least one antibody according to any one of claims 21-23.

27. The immunogenic or vaccine composition of claim 26, which further comprises at least one polypeptide comprising the sequence of SEQ ID NO: 14 or an ortholog thereof in a *Plasmodium* species selected from *P. vivax, P. ovale, P. malariae, P. berghei, P. knowlesi, P. chabaudi, P. yoelii.*

28. The antibody of any one of claims 21-23, for use in the diagnosis of malaria.

29. A nucleic acid, coding for a polypeptide according to any one of claims 1-16.

30. A vector, comprising an insert consisting of a nucleic acid according to claim 29.

31. A genetically engineered host cell comprising a nucleic acid according to claim 29, and/or a vector according to claim 30.

32. A pair of oligonucleotide primers, which specifically prime the amplification of an amplicon consisting of a nucleic

acid according to claim 29.

33. An oligonucleotide probe, which specifically hybridizes to a nucleic acid according to claim 29 under stringent conditions.

```
LOCUS       XM_960931          3312 bp    mRNA    linear   INV 07-FEB-2007
DEFINITION  Plasmodium falciparum 3D7 hypothetical protein (PFF0165c) mRNA,
            complete cds.
ACCESSION   XM_960931
VERSION     XM_960931.1  GI:86170479
KEYWORDS    .
SOURCE      Plasmodium falciparum 3D7
  ORGANISM  Plasmodium falciparum 3D7
            Eukaryota; Alveolata; Apicomplexa; Aconoidasida; Haemosporida;
            Plasmodium; Plasmodium (Laverania).
REFERENCE   1  (bases 1 to 3312)
  AUTHORS   Cherevach,I., Davis,P., Goodhead,I., Stevens,K., Mungall,K.,
            Berry,A.E., Berriman,M., RA Pain,A., Hall,N., Atkin,R.,
            Chillingworth,C., Doggett,J., Ormond,D., Sanders,M., Hayes,R.,
            Hall,S., Quail,M. and Barrell,B.G.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-MAR-2004) P.falciparum Genome Sequencing
Consortium,
            The Welcome Trust Sanger Institute, Wellcome Trust Genome
Campus,
            Hinxton, Cambridge CB10 1SA, UK
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to
final
            NCBI review. This record is derived from an annotated genomic
            sequence (NC_004327).
            COMPLETENESS: incomplete on both ends.
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FIGURE 1 (start)

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FIGURE 1 (continued)

ORIGIN

```
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      361 aacaataata tgattaaaga agatcttgaa gatgatgcca atcagaatat tttgatgaaa
      421 tcaccttatt ataatataga aaatttttta caagtttttt taaaatatat taataagaag
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      661 ttgtatcata ataacaatga aaagaatata tcatatgata aaaatttagt taaacaagaa
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      781 aatgagagag gggaaatgtt agataagggt aaatcttatt caggtgacga aaaaataaat
      841 acaagtgata atgctaaatc atgttcaggt gacgaaaaag taattacaag cgataatggt
      901 aaatcttatg attatgtaaa aaatgaaagt gaagaacaag aagaaaaaga aaatatgtta
      961 aataataaaa aaagaagttt ggaatgtaat ccaaatgaag cgaaaaaaat ttgtttctct
     1021 ttagaagaga agataggaac tgtgcaaagt gtaaaattaa aggaatataa tgaattgagt
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     1201 aattatagaa atgaagaaga gaaaaaaaaa aatcaaataa attttgatta tttaaaaaaa
     1261 aaaattaaga ataaccaaga tgtttttgag gaaacgatac aaaaatgttt tttgataaat
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     1441 tatattgata taaaaaagaa aataagcgaa ttacaaaagg ataacgaaag tttaaaaatt
     1501 caggtagata ggctagagaa aaagaaggct acattaatat acaaattgaa taatgataat
     1561 attcgtaaac atattcttga taataatatt aaagattatc aaaatggtat tgataattca
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     1741 aatagtgatg ataattataa tagtgaggat aatgaatata ataatggtaa ttatcgattt
     1801 cgtaataatt ataagaagga ttctttgaat gaagatgatg taaaaaaaaa tcctttgaag
     1861 gtatgtcaca aaattaacag tgattctaat atttttgtta attttgaaaa tattataaca
     1921 aaacaaaata ttatacatag tgaaccattt cgaaatttat taaaagaatc taatgaatta
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     2881 caaatgaatt tatttataaa attaaaagat aaatatgata tcatatttca aaaaaaaata
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     3001 caagatttag aaaaagaaaa ttctactctt cagaataaac tatctaacga aataaagaat
     3061 tcaaaaatgc tatccaaaaa tttatctaaa aattctgatg atcatttatt aattgaagaa
     3121 aataatgaat taagaagaag attaatttgt agtgtatgta tggaaaactt tagaaattat
     3181 attattatca aatgtggtca tatttattgt aacaattgta tattcaataa tttaaaaaca
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     3301 tttctcgact aa
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(SEQ ID NO: 1)

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FIGURE 1 (continued)

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DEFINITION  hypothetical protein PFF0165c [Plasmodium falciparum 3D7].
ACCESSION   XP_966024
VERSION     XP_966024.1  GI:86170480
DBSOURCE    REFSEQ: accession XM_960931.1
KEYWORDS    .
SOURCE      Plasmodium falciparum 3D7
  ORGANISM  Plasmodium falciparum 3D7
            Eukaryota; Alveolata; Apicomplexa; Aconoidasida; Haemosporida;
            Plasmodium; Plasmodium (Laverania).
REFERENCE   1  (residues 1 to 1103)
  AUTHORS   Cherevach,I., Davis,P., Goodhead,I., Stevens,K., Mungall,K.,
            Berry,A.E., Berriman,M., RA Pain,A., Hall,N., Atkin,R.,
            Chillingworth,C., Doggett,J., Ormond,D., Sanders,M., Hayes,R.,
            Hall,S., Quail,M. and Barrell,B.G.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-MAR-2004) P.falciparum Genome Sequencing
Consortium,
            The Welcome Trust Sanger Institute, Wellcome Trust Genome
Campus,
            Hinxton, Cambridge CB10 1SA, UK
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to
final
            NCBI review. The reference sequence was derived from CAG25204.
FEATURES             Location/Qualifiers
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                     /isolate="3D7"
                     /db_xref="taxon:36329"
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     Protein         1..1103
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                     /calculated_mol_wt=132340
     Region          1050..1092
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                     /note="RING-finger (Really Interesting New Gene)
domain, a
                     specialized type of Zn-finger of 40 to 60 residues
that
                     binds two atoms of zinc; defined by the 'cross-brace'
                     motif C-X2-C-X(9-39)-C-X(1-3)-
                     H-X(2-3)-(N/C/H)-X2-C-X(4-48)C-X2-C; probably involved
in
                     medi; cd00162"
                     /db_xref="CDD:29102"
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FIGURE 1 (continued)

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                     /old_locus_tag="MAL6P1.37"
                     /coded_by="XM_960931.1:1..3312"
                     /note="Putative Zn-finger motif at the C-terminus"
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                     /db_xref="HSSP:P25916"
                     /db_xref="InterPro:IPR001841"
                     /db_xref="UniProtKB/TrEMBL:Q6LFN2"
                     /db_xref="GeneID:3885786"
ORIGIN
        1 msnkkrskne ndestslple nsellieyih nlksclnvyr reiqeknkyi siikndlsfh
       61 eciltnvnvv wsvfnndlln llcnneqkee geeiikqrni gdeineynnl tklqndenik
      121 nnnmikedle ddanqnilmk spyynienfl qvflkyinkk kkkvkvkvkd egkkekiedk
      181 kyeqddeeen eeeeeeeeee egeeenkede effktfvsfn lyhnnnekni sydknlvkqe
      241 ndnkdeargn dnmcgnydih nergemldkg ksysgdekin tsdnakscsg dekvitsdng
      301 ksydyvknes eeqeekenml nnkkrslecn pneakkicfs leekigtvqs vklkeynels
      361 kenieknkhd dnnicnylsh negenviere dklfnklnnk nyrneeekkk nqinfdylkk
      421 kiknnqdvfe etiqkcflin lkktlnlink imylknvefr kynldyirki nyekcfyykn
      481 yidikkkise lqkdneslki qvdrlekkka tliyklnndn irkhildnni kdyqngidns
      541 kvsyfdegen pynrnnknyr tdnknsddnn nnnnyyynny nsddnynsed neynngnyrf
      601 rnnykkdsln eddvkknplk vchkinsdsn ifvnfeniit kqniihsepf rnllkesnel
      661 yitlkekeke niilkneilk menkkdeeye hllnntiedk keltrsikel einmmtcnme
      721 kdkisnkvnt leyeinvlkn idknqtmqlq qkendilkmk lyieklklse knlkdkiill
      781 enekdkmlsg ihikdnsfne eskseegkiq lrdiqndnde kyddekkrfk elfienqklk
      841 eelnkkrnve eelhslrkny niineeieei tkefekkqeq vdemilqikn kelelldkfn
      901 nkmnkayvee klkelkntye ekmkhinniy kkhddfvniy lnlffqarkn ailsdsqree
      961 qmnlfiklkd kydiifqkki eltdilknvy dcnkklighc qdlekenstl qnklsneikn
     1021 skmlsknlsk nsddhlliee nnelrrrlic svcmenfrny iiikcghiyc nncifnnlkt
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FIGURE 1 (end)

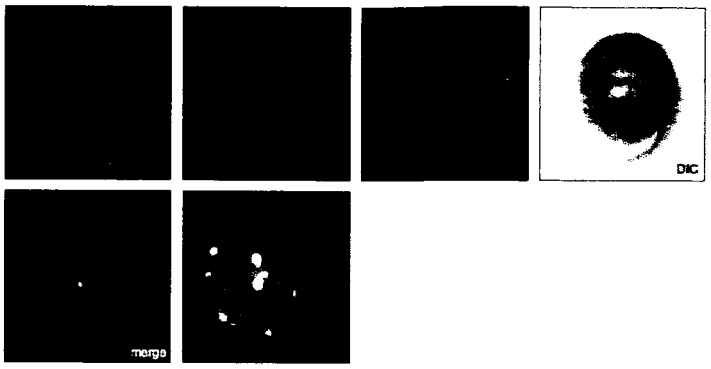


FIGURE 2

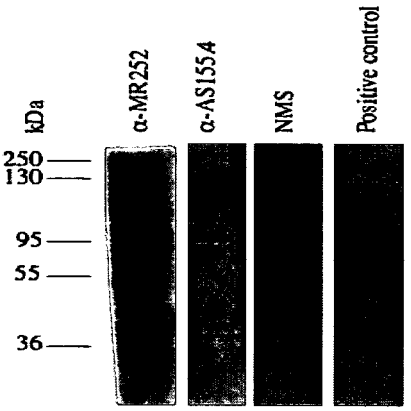
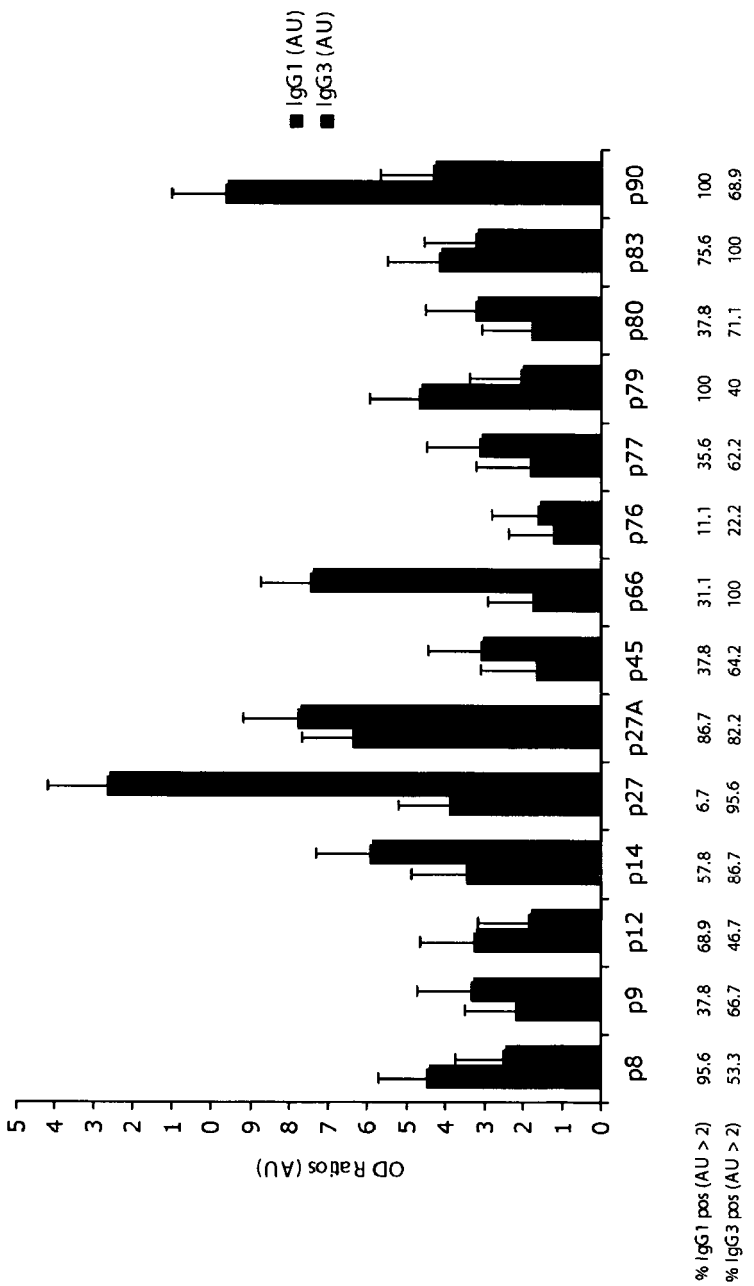


FIGURE 3


IgG1 (AU)
IgG3 (AU)
OD Ratios (AU)
0
1
2
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4
5
6
7
8
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p27
p27A
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p66
p76
p77
p79
p80
p83
p90
% IgG1 pos (AU > 2) 95.6 37.8 68.9 57.8 6.7 86.7 37.8 31.1 11.1 35.6 100 37.8 75.6 100
% IgG3 pos (AU > 2) 53.3 66.7 46.7 86.7 95.6 82.2 64.2 100 22.2 62.2 40 71.1 100 68.9

FIGURE 4

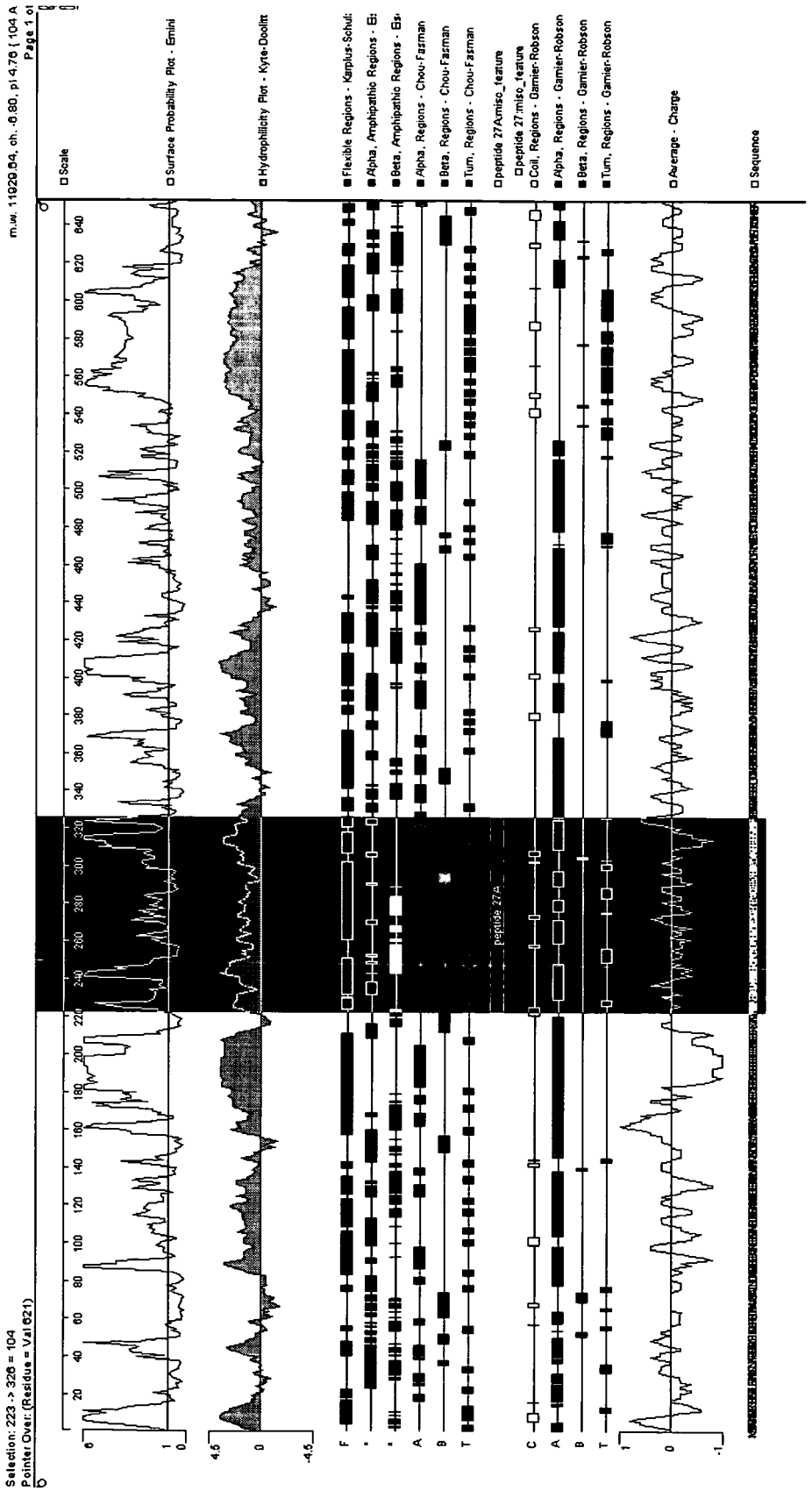

FIGURE 5 (start)

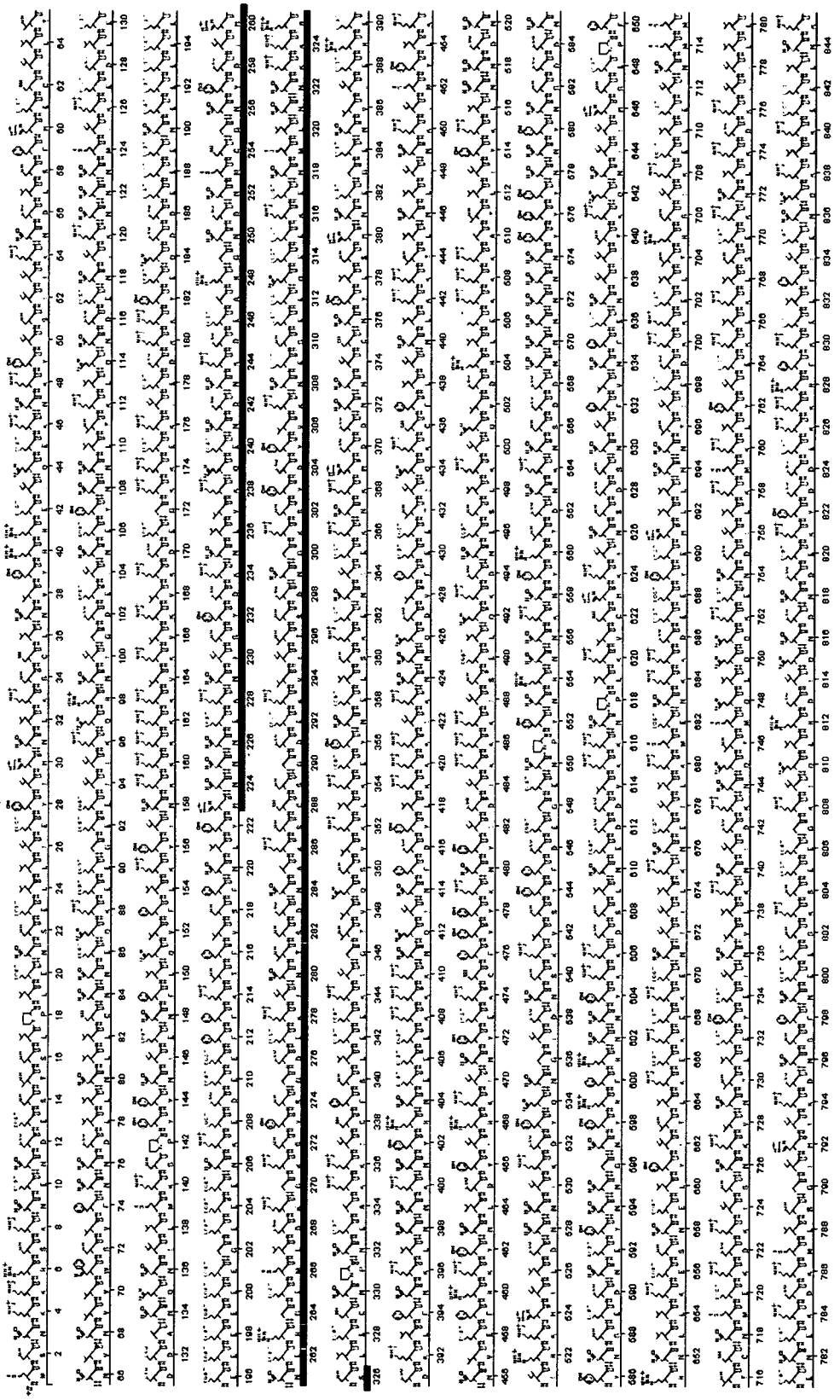

FIGURE 5 (end)

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EUROPEAN SEARCH REPORT

Application Number
EP 08 29 0433

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	EP 1 754 717 A (UNIV LAUSANNE [CH]) 21 February 2007 (2007-02-21)	1,3,4, 6-12, 17-33	INV. A61K39/395 C07K16/20
A	0012-0020; p. 4, l. 19; p. 7, lines 8-9; p. 11, line 48; 0045; 0093-0096; example 3; table 3; example 5; claims	2,5, 13-16	C07K14/445 A61P33/06
A	HALL N ET AL: "Sequence of Plasmodium falciparum chromosomes 1, 3-9 and 13" NATURE, NATURE PUBLISHING GROUP, LONDON, UK, vol. 419, no. 6906, 3 October 2002 (2002-10-03), pages 527-531, XP002277348 ISSN: 0028-0836 * the whole document *	1-33	
A	CORRADIN GIAMPIETRO ET AL: "Protein structure based strategies for antigen discovery and vaccine development against malaria and other pathogens" ENDOCRINE, METABOLIC & IMMUNE DISORDERS - DRUG TARGETS, BENTHAM SCIENCE PUBLISHERS LTD, BUSSUM, NL, vol. 7, no. 4, 1 December 2007 (2007-12-01), pages 259-265, XP009107409 ISSN: 1871-5303 * the whole document *	1-33	

-/--

TECHNICAL FIELDS SEARCHED (IPC)
C07K
A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	20 November 2008	Renggli, John

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EUROPEAN SEARCH REPORT

Application Number
EP 08 29 0433

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	BALLOU W R ET AL: "UPDATE ON THE CLINICAL DEVELOPMENT OF CANDIDATE MALARIA VACCINES" AMERICAN JOURNAL OF TROPICAL MEDICINE & HYGIENE, AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE, US, vol. 71, no. 2, SUPPL. 02, 1 August 2004 (2004-08-01), pages 239-247, XP009057966 ISSN: 0002-9637 * the whole document * -----	1-33	
			TECHNICAL FIELDS SEARCHED (IPC)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	20 November 2008	Renggli, John

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		WO 2007020520 A2	22-02-2007

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